2.0 THE INFLUENCE OF EXTERNAL CATION CONCENTRATION ON THE HATCHING OF AMPHIBIAN EMBRYOS IN WATER OF LOW PH

2.1 INTRODUCTION

Amphibians are most sensitive to low pH during embryonic development (Pierce *et al*. 1984). An understanding of how acidic water and its various components affects embryos is essential to proper evaluation of the relation of pond pH to natural patterns of species distribution and abundance. Gosner and Black (1957) were the first to demonstrate interspecific differences in tolerance of amphibian embryos to low pH. They reported that the most sensitive species were restricted to waters high in pH within the generally acidic New Jersey Pine Barrens. Saber and Dunson (1978) found that many locally abundant amphibians were absent from a naturally acidic sphagnum bog in Central Pennsylvania. Beebee and Griffin (1977) observed that the natterjack toad (*Bufo clamita*) did not breed in acidic ponds which would kill their eggs in heathland areas of England. In central Pennsylvania, the wood frog (*Rana sylvatica*) could breed in virtually all available ponds owing to their high tolerance to low pH. The Jefferson salamander (*Ambystoma jeffersonianum*) was absent from the most acidic ponds and their embryos were

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Report No. 22

THE EFFECT OF ACIDIC PRECIPITATION ON AMPHIBIAN BREEDING IN TEMPORARY PONDS IN PENNSYLVANIA

by

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USDI FWS Coop. Agreement #14-16-0009-154B

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Washington, D.C. 20240

Fish and Wildlife Service

U.S. Department of the Interior

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UNITED STATES
DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

EASTERN ENERGY AND LAND USE TEAM
Box 705
Kearneysville, West Virginia 25430

Dear Colleague:

The Eastern Energy and Land Use Team (EELUT) is pleased to provide you this report which assesses the impact of acidic deposition on amphibians which breed in temporary ponds in Pennsylvania. This study is part of the continuing efforts of the Fish and Wildlife Service to better define and understand the influence of acid deposition on fish and wildlife resources. This is the twenty-second report in our series dealing with air pollution and acid rain. Previous reports in this series are listed on the following page.

This research project included investigation of the lowest pH's at which embryos of certain amphibian species could hatch, and the influence of external ion concentration on embryonic tolerance; the physiological effects of low pH on amphibian larvae; pond chemistry and the influence of rainfall on pond pH and other chemical variables; and the effect of pond pH on embryonic survival and how these factors influence the local distribution.

We are interested in your comments concerning this publication and invite you to write or call us (phone 304/725-2061; FTS 925-5279).

Sincerely,

R. Kent Schreiber

R. Kent Schreiber
Acting Team Leader, EELUT

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DISCLAIMER

Although the research described in this report has been funded in part by the U.S. Environmental Protection Agency through Interagency Agreement No. EPA-82-D-X0581 to the U.S. Fish and Wildlife Service, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency. This research has been funded as part of the National Acid Precipitation Assessment Program by the U.S. Fish and Wildlife Service. Support was also obtained from the Pennsylvania State University Fund for Academic Excellence. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the Federal Government.

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EXECUTIVE SUMMARY

In order to assess the impact of acidic deposition on amphibians which breed in temporary ponds in Pennsylvania, we investigated: 1) the lowest pH's at which embryos of each species could hatch, and the influence of external ion concentration on embryonic tolerance; 2) the physiological effects of low pH on amphibian larvae; 3) pond chemistry and the influence of rainfall on pond pH and other chemical variables; and 4) the effect of pond pH on embryonic survival and how these factors influence the local distribution of Jefferson salamanders (*Ambystoma jeffersonianum*) and wood frogs (*Rana sylvatica*).

Amphibian embryos exposed to water of low pH were killed by two distinct mechanisms. At very low pH's, embryos stopped development soon after exposure to test solutions. At higher but still lethal pH's, embryos became curled within a shrunken perivitelline space and failed to hatch (curling defect). The addition of Ca, Mg, and to a lesser extent Na (> 10 mg/l), prevented the early mortality of embryos in acidic water. However, increasing concentrations of these ions (Mg > Ca > Na) also caused the curling defect. Embryos of *Ambystoma* were generally able to hatch even though they became curled, but *R. sylvatica* remained trapped and died. In consequence, as the concentration of Ca, Mg, or Na was increased at low pH, greater numbers of embryos of *Ambystoma* hatched, while survival of embryos of *R. sylvatica* was drastically reduced.

Sodium influx, efflux, and body ionic contents of amphibian larvae were measured during acute and chronic exposures to acidic and neutral waters. Acute exposure to low pH's (2.5-4.0) depressed sodium influx and markedly accelerated sodium efflux. The resulting net loss of 50% of body sodium was fatal. Increasing the external calcium concentration extended survival time by slowing the loss of sodium. Chronic exposure to low pH caused a 21 to 62% reduction in body sodium content; potassium content did not change. Chronic exposure also caused sodium influx to increase and efflux to decrease in comparison to fluxes measured during acute exposure at the same pH. Interspecific sensitivity differences were related to the relative magnitude of disruption in sodium balance. The three species of tadpoles studied differed in body sodium content under control conditions. Initial body sodium content was inversely correlated with acid tolerance and may represent a previously unknown mechanism for coping with physiologically stressful low pH environments.

The effect of sublethal, chronic exposure of tadpoles to low environmental pH under natural conditions was investigated by making weekly measurements of various physiological variables. *Rana sylvatica* tadpoles from a low pH pond (pH 4.5-4.90) had lower body sodium, chloride, and water concentrations in comparison with tadpoles from a nearby high pH pond (pH 5.74-6.37). Tadpoles from both ponds placed in the low pH pond also underwent higher sodium efflux than when placed in the high pH pond. These observations parallel similar studies on fish and demonstrate the effects of low environmental pH on larval anuran ionic regulatory mechanisms. Survival times of field-collected *R. sylvatica* tadpoles placed at pH 3.0 (H_2SO_4) did not change with age nor were there any differences between tadpoles from high and low pH ponds. However, survival times of laboratory raised *R. pipiens* tadpoles did increase with age.

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In central (39 ponds surveyed) and northeastern Pennsylvania (35 ponds surveyed), 15 and 11% of amphibian breeding sites, respectively, were acidic enough (less than pH 4.5) to cause direct mortality of *Ambystoma* salamander embryos. In both areas, an additional 16% of ponds were low enough in pH (pH 4.5-5.0) to cause sublethal effects such as larval growth inhibition. Aluminum concentration (total dissolved) was negatively correlated with pond pH and reached 0.60 mg/l at pH 4.20. We were unable to determine the relative contributions of acidic precipitation and that of natural factors (sphagnum, organic acids, etc.) to the acidity of low pH ponds. It is, however, quite clear that our study ponds were extremely vulnerable to acidification by rain because of their very low alkalinity. We suggest further studies to determine the exact source of acidity in low pH ponds and further monitoring designed to detect future declines in pH.

The effect of low environmental pH on amphibians was studied in twelve temporary forest ponds of central Pennsylvania. The salamander, *A. jeffersonianum*, was intolerant of low pH and was absent from the most acidic ponds in Pennsylvania. The wood frog, *R. sylvatica*, was tolerant and occurred in ponds with the lowest pH's. Laboratory tests demonstrated that *A. jeffersonianum* could not hatch below pH 4.50, whereas *R. sylvatica* could hatch even at pH 4.25. Embryos of both species were transplanted into several ponds ranging in pH from 4.10 to 7.25 to test whether embryonic mortality caused by low pH was responsible for the absence of *A. jeffersonianum* from acidic ponds. Hatching of embryos of *R. sylvatica* was not related to pond pH, while mortality of embryos of *A. jeffersonianum* increased significantly as pond pH declined. In laboratory trials, tadpoles of *Bufo woodhousei* and *Hyla andersoni* grew significantly slower than high pH controls when exposed to low pH. This sublethal effect on body size has important implications for dynamics of amphibian populations in acidic ponds.

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1.0 INTRODUCTION

Salamanders and frogs can be arboreal, terrestrial, subterranean, semi-aquatic, or totally aquatic. However, a common feature of the natural history of most species is reproduction in aquatic habitats. Embryonic and larval development generally takes place in water, with the exception of a few species of plethodontid salamanders which are totally terrestrial. Amphibians can breed in lakes, ponds, streams, bogs, and even puddles. Temporary forest ponds are heavily utilized by amphibians for reproduction. Within the United States, 50% of the species of frogs and toads, and one-third of all salamander species breed in temporary forest ponds (Pough and Wilson, 1977). Many species, including the wood frog (Rana sylvatica) and Ambystoma salamanders, are specialized and will only use this type of pond. Temporary forest ponds could be among the most susceptible bodies of water to acidification by rain, because of their complete refilling each year by rainfall and snow melt. These ponds are usually dry by the end of summer, and acidic rain entering the pond during early spring is not diluted. Pough (1976) and Cook (1978, 1983) observed maximum pH depressions of 0.25 and 0.75 immediately after large rainfalls. Adult amphibians return to ponds during March and April to mate and lay eggs. Deciduous trees have not yet sprouted leaves, so rain is not buffered in the canopy. Amphibian reproduction may be seriously jeopardized since pond pH's are lowest in early spring when embryos, the most sensitive stage, are present (Chapter 4).

When this project was initiated, little or no information existed regarding many aspects of the effects of low environmental pH on amphibians. Due to the paucity of information, we took a broad approach to this problem, and investigated toxicological, physiological and ecological questions. We studied the effect of external ion concentration on hatching of embryos in low pH water in order to facilitate comparisons between different studies and to help extrapolate to field situations. We investigated the effect of water low in pH on ionic regulation in tadpoles, because previous studies have shown that the cause of death for fish in acidic water is salt (NaCl) loss (McDonald, 1984). Descriptive studies of the chemistry of temporary ponds were initiated and transplantation of embryos was performed to determine if embryos suffered mortality in acidic ponds. Each of these topics will be discussed separately in the following chapters.

2.0 THE INFLUENCE OF EXTERNAL CATION CONCENTRATION ON THE HATCHING OF AMPHIBIAN EMBRYOS IN WATER OF LOW PH

2.1 INTRODUCTION

Amphibians are most sensitive to low pH during embryonic development (Pierce _et al_. 1984). An understanding of how acidic water and its various components affects embryos is essential to proper evaluation of the relation of pond pH to natural patterns of species distribution and abundance. Gosner and Black (1957) were the first to demonstrate interspecific differences in tolerance of amphibian embryos to low pH. They reported that the most sensitive species were restricted to waters high in pH within the generally acidic New Jersey Pine Barrens. Saber and Dunson (1978) found that many locally abundant amphibians were absent from a naturally acidic sphagnum bog in Central Pennsylvania. Beebee and Griffin (1977) observed that the natterjack toad (_Bufo clamita_) did not breed in acidic ponds which would kill their eggs in heathland areas of England. In central Pennsylvania, the wood frog (_Rana sylvatica_) could breed in virtually all available ponds owing to their high tolerance to low pH. The Jefferson salamander (_Ambystoma jeffersonianum_) was absent from the most acidic ponds and their embryos were killed when transplanted into them (Chapter 5).

Recent declines in pH of some aquatic habitats have been attributed to acidic precipitation. Pough and Wilson (1977) and Cook (1983) reported that rainstorms depressed the pH of temporary ponds by 0.25 to 0.75 units. Pough and Wilson (1977) observed high natural mortality of naturally laid embryos of _A. maculatum_ in New York, and they predicted a bleak future for _Ambystoma_ salamanders in northeastern North America. In contrast, Cook (1983) reported low mortality of naturally laid embryos in a pH 4.25 pond in the Connecticut Valley of Massachusetts. Acid mine drainage and acidic leachates from geological formations exposed by road cuts are also known to kill amphibian embryos (Porter and Hakanson, 1976; Mathews and Morgan, 1982).

Embryos of amphibians are more sensitive to low pH than are larvae (Pierce _et al_., 1984; Dale _et al_., 1984; Chapters 2 and 3). Thus reproductive failure of amphibians in acidic ponds is therefore usually a result of embryonic mortality. Embryos killed by low pH usually exhibit characteristic abnormalities. The most common of these is a tight curling of embryos within a shrunken vitelline membrane (Gosner and Black, 1957; Pough and Wilson, 1977). Other deformities include swelling of the thoracic region and failure to retract the yolk plug (Pough and Wilson, 1977; Chapters 2 and 5). Dunson and Connell (1982) found that embryos did not become curled and survived in acidic water if the vitelline membrane was removed. This clearly indicates that the mortality associated with the curling defect is caused by membrane dysfunction rather than a general toxic effect on the embryo itself. Dunson and Connell (1982) hypothesized that low pH inhibited the activity of the hatching enzyme and/or other mechanisms responsible for the enlargement of the perivitelline space.

Embryonic tolerance of different species to low pH has been measured repeatedly in the laboratory but with somewhat conflicting results (Tome and Pough, 1982; Pierce _et al_., 1984; Dale _et al_., 1984; Chapter 2). Similarly, field studies in different geographic locations have reported varying levels of mortality in acidic ponds of comparable pH's (Pough, 1976; Cook, 1983; Chapter 5). These differences in experimental results are often attributed to inter-population genetic variation. In one such study, intraspecific variations in tolerance to low pH have been shown to be genetically controlled (Pierce and Sikland, 1985). However, comparisons of tolerance between studies may be misleading, since experimental protocols (especially composition of test water) are not comparable. The concentrations of cations, especially calcium, can strongly influence survival of tadpoles in acidic water (Chapter 3). Clark and Hall (1985) have demonstrated that aluminum can significantly lower hatching of amphibian embryos in low pH water. The main objective of the present study was to evaluate the effects of different cations (Na, Mg, Ca) on hatching of amphibian embryos in low pH water. These data may assist in interpreting results of laboratory studies which have used chemically different incubation solutions, and of field observations in ponds of differing chemical composition.

2.2 METHODS

Source of Embryos - Embryos of *R. sylvatica*, *A. maculatum*, *A. jeffersonianum*, *Bufo woodhousei*, and *B. americanus* were collected from temporary ponds in Centre County, Pennsylvania. Egg masses were collected within 24 hours of oviposition and embryos were usually at stage 4 (4 cell stage) or younger (Gosner, 1960). Table 1 lists the chemical composition of pond water from which eggs were removed. Water analyses were performed according to APHA *et al.* (1975). Embryos of the leopard frog (*Rana pipiens*) were obtained from artificial mating (Rugh, 1952) of commercially purchased adults (Nasco, Inc.). Eggs were fertilized and maintained in artificial soft water (ASW, Table 1) until transfer to experimental solutions.

Experimental Solutions - Solutions were prepared by diluting specific quantities of 1000 mg/L stock solutions (NaCl, KCl, $MgCl_2$, and $CaCl_2$) to 1 L with distilled water. Except for the ion which was varied, the concentration of all other cations was 1 mg/L; Mg was not added to experimental solutions in which Ca or Na were varied. One liter aliquots of test water were placed in glass culture dishes 20 cm in diameter. Solutions were analyzed before embryos were added and after they hatched. Concentrations of cations changed little during the duration of experiments ($<$ 5%). The pH was adjusted with dilute H_2SO_4 and rarely drifted by more than 0.05 units per day. The pH was checked daily and adjusted when it drifted by more than 0.05 units from original values. Embryos of *Ambystoma* and *R. sylvatica* were kept in an environmental chamber at 9 C (± 1 C), under a 12:12 photoperiod. Embryos of *R. pipiens* and *Bufo* spp. were maintained at room temperature (20-22 C) and ambient illumination.

Biological Measurements - Twelve egg masses of *A. jeffersonianum*, *A. maculatum*, and *R. sylvatica* were collected and returned to the laboratory in their native pond water (within 1 h). Egg masses of *A. jeffersonianum* and *R. sylvatica* were cut into pieces containing approximately 5 to 7 embryos. Embryos of *A. maculatum* surrounded by their vitelline membrane were individually removed from their egg masses because of the opaque nature of the outer jelly. Tome and Pough (1982) reported no difference in hatching between

Table 1. Chemical composition of ponds where amphibian egg masses were collected, and of artificial soft water (ASW) used in laboratory tests.

				(C)			(mg/L)		
Species	Date	Test	pH	Temp	Na	K	Mg	Ca	Cl
Rana sylvatica	March 16, 1983	Ca	5.00						
	April 4, 1983	Ca	4.20	5	0.3	0.5	0.5	1.2	0.7
	April 4, 1984	Mg & Na	4.20	6	0.3	0.6	0.4	1.1	1.1
Ambystoma jeffersonianum	March 14, 1983	Ca	4.72	9	0.6	1.1	0.8	2.5	
	March 26, 1984	Mg & Na	4.62	8	0.4	0.6	0.5	1.2	1.4
Ambystoma maculatum	March 21, 1983	Ca	4.85	5	0.4	0.2	0.8	1.6	
Bufo woodhousei	June 6, 1982	Ca	7.40	15					
Bufo americanus	May 1, 1983	Ca	6.13	15					
ASW			5.80		1.0	1.0	0	1.0	4.2

embryos maintained in or removed from the egg jelly. Individuals or groups of B. americanus and B. woodhousei were from the same clutch and egg strings were cut into segments containing the correct number of embryos (n=20 and n=10 embryos per bowl, respectively). All embryos of R. pipiens were from the same clutch; sample size was 10 embryos per bowl. Since embryos of any one species were removed from a single pond or an individual clutch, the results should be interpreted cautiously because much variability in tolerance to low pH has been documented (Pierce & Sikland, 1985). This condition does however reduce variability and makes interpretation of the effects of cations clearer, which was the objective of these experiments.

The developmental stages of the embryos were determined daily and the number of live and dead animals recorded. Descriptions of dead or dying embryos and notes concerning the remaining live embryos were also made. Dead embryos were not removed, mimicing field conditions. The diameter of the perivitelline space of embryos of A. jeffersonianum (n=5) at stage 20 was measured in experimental solutions of Mg and Na with an ocular micrometer. The degree of embryonic curling is directly proportional to the diameter of the perivitelline space. Embryos were scored as hatched if they completely escaped from their membranes, and hatchlings were classified as deformed if they exhibited gross morphological abnormalities.

Chi square tests were performed to detect significant effects of either pH or ion concentration on hatching of each species ($\alpha = 0.05$). Page's one way test (Daniel, 1978) for ordered alternatives determined whether ion concentrations or pH had a significant effect on the diameter of the perivitelline space ($\alpha = 0.05$).

2.3 RESULTS

2.3.1 Effects of Calcium

Ambystoma jeffersonianum - Calcium concentration had a significant effect on hatching at pH 4.0 and 4.5 (Table 2). At pH 4.5 and 1 mg/L Ca, all embryos became tightly curled within their shrunken vitelline membrane. Thirty percent were killed by this membrane constriction while the remaining embryos managed to hatch, but had severely bent spines. At 10 and 20 mg/L (pH 4.5), embryos appeared normal and all hatched; those in 40 and 80 mg/L became severely curled, but still managed to hatch. These constricted embryos were able to hatch by forcing themselves through a small hole which formed in the vitelline membrane immediately in front of the head. This process was lengthy and some hatchlings remained half-emerged with their mid-section tightly constricted for up to 24 hours. Embryos kept at pH 4.0 and 1 and 10 mg/L Ca stopped developing shortly after the start of the experiment. Embryos at 20 to 80 mg/L did survive until hatching, but became curled within their egg membrane. No embryos hatched at 20 mg/L, but 20 and 40% did escape at 40 and 80 mg/L, respectively. Curling was much more severe at pH 4.0 where embryos were constricted into a spherical shape. Embryos had a ring-like appearance at pH 4.5. Between 95 and 100% of embryos incubated in pH 5.8 and 5.0 water hatched and none became curled.

Table 2. Percent hatching and cause of death in embryos and occurrence of deformities in hatchlings of *Ambystoma jeffersonianum* at various pH's and concentrations of calcium (n = 20 embryos per bowl).

pH	Ca (mg/L)	% Hatched	% of hatchlings deformed	% of embryos killed early	by curling
5.8	1	100	0	0	0
	10	95	0	5	0
	20	95	0	5	0
	40	100	0	0	0
	80	100	0	0	0
5.0	1	100	0	0	0
	10	100	0	0	0
	20	95	0	5	0
	40	100	0	0	0
	80	95	0	5	0
4.5	1	70	100	0	30
	10	100	0	0	0
	20	100	5	0	0
	40	100	0	0	0
	80	100	0	0	0
4.0	1	0	0	100	0
	10	0	0	100	0
	20	0	0	0	100
	40	20	0	5	75
	80	40	50	5	55

Ambystoma maculatum - The response to Ca was generally similar to that of *A. jeffersonianum* but the low hatching in controls (pH 5.8) make interpretation of the results more difficult. Hatching was elevated (α= 0.10) at pH 4.0 when Ca was increased (Figure 1). At pH 4.0, with 1 and 10 mg/L Ca, 70 to 95% embryos stopped developing soon after exposure. At higher concentrations of Ca (20-80 mg/L), 45 to 55% of the embryos died early, but the survivors suffered from the curling defect. The degree of curling at pH 4.0 was directly related to the concentration of Ca. Embryos at 1 and 10 mg/L had the head and tail touching, while those at 40 and 80 mg/L were compressed into tight spheres. No curling occurred at any concentration of Ca at pH's 4.5, 5.0, and 5.8.

Rana sylvatica - In contrast to the previous two cases, Ca significantly increased mortality of this anuran at pH 4.5 (Table 3). The addition of Ca (20-80 mg/L) significantly reduced early mortality at pH 4.0. However, embryos became progressively more curled at each pH as the concentration of Ca increased and were forced into a spherical shape at 40 and 80 mg/L. At pH 5.8 and 5.0 (40-80 mg/L Ca), embryos hatched through a small hole in the vitelline membrane as previously described. Embryos at pH 4.5 and 4.0 (40-80 mg/L) never escaped and died.

Bufo woodhousei, Bufo americanus, and Rana pipiens - Hatching of these three species was significantly increased at low pH by the addition of Ca, but the response for each species was quite different (Figure 2). High concentrations of Ca (> 10 mg/L) at pH 4.0 reduced death of *B. americanus* during initial stages of development, without increased membrane constriction. Fifteen to 67% of hatchlings at pH 4.00 had swollen thoracic regions. Slight membrane constriction did occur at pH 4.25 with 40 to 80 mg/L Ca, resulting in a few hatchlings with bent spines. Embryos of *B. woodhousei* underwent no curling and were able to hatch at pH 4.00 if Ca was added to the incubation media. Embryos of *R. pipiens* kept in pH 4.5 water with 1 mg/L Ca died from membrane constriction, while those at 10 to 40 mg/L lived and did not become curled. Embryos in 80 mg/L did become curled but were able to hatch. At pH 4.0 and 1 to 20 mg/L Ca, all embryos died shortly after exposure, while approximately 50% in 40 and 80 mg/L survived until just before hatching, at which time most were killed by membrane constriction.

2.3.2 Effects of Magnesium

Ambystoma jeffersonianum - The effect of Mg on embryos was similar to that of Ca (Figures 3 and 4). At pH 4.25, the number of embryos killed early at each concentration of Mg was not significantly different. Thus, the significantly different levels of hatching reflected the ability of embryos to escape from the shrunken vitelline membrane. At pH 4.00, all embryos in 1 and 10 mg/L Mg died during the first few days of exposure, while between 70 and 95% of the embryos in 20 to 80 mg/L survived until near hatching, but were then killed by the curling defect. The perivitelline space of embryos at pH's of 5.80, 4.75, and 4.50 became significantly reduced as the concentration of Mg increased (Figure 4). Increasing the concentration of Mg did not significantly reduce the diameter of the perivitelline space at pH 4.25, since embryos were already maximally constricted at 1 mg/L. The volume of the

AMBYSTOMA MACULATUM

pH	1	10	20	40	80
5.8	70	75	70 (7)	65 (8)	80
5.0	45	55	45	35	60
4.5	60	50	60	60	75
4.0	5	20	35	30	35 (14)

[CALCIUM], Mg/L

Figure 1. Percent hatching of embryos of Ambystoma maculatum (n=20) exposed to various pH's and concentrations of calcium. Numbers in parentheses indicate percent of hatchlings which were deformed (no parentheses = no deformities.

Table 3. Percent hatching and cause of death in embryos and occurrence of deformities in hatchlings of *Rana sylvatica* at various pH's and concentrations of calcium (pH 4.0, 4.5, and 5.8, 2 replicates of n=20; pH 5.0, 1 replicate of n=20).

pH	Ca (mg/L)	% Hatched	% of hatchlings deformed	% of embryos killed	
				early	by curling
5.8	1	95	0	0	5
	10	95	21	2	3
	20	100	23	0	0
	40	95	16	0	5
	80	98	13	0	0
5.0	1	100	0	0	0
	10	95	0	5	0
	20	80	0	20	0
	40	100	10	0	0
	80	100	0	0	0
4.5	1	90	3	10	0
	10	78	3	2	10
	20	100	0	0	0
	40	33	31	5	62
	80	15	67	20	65
4.0	1	10	75	58	32
	10	13	20	35	52
	20	13	20	7	80
	40	8	0	15	77
	80	0	0	10	90

BUFO AMERICANUS

pH	1	10	20	40	80
5.50	100	100	100	100	100
5.00	100	100	100 (5)	100	100
4.50	100 (5)	100	100	100	100
4.25	90	100	100	95 (16)	75 (20)
4.00	0	10 (50)	30 (67)	65 (15)	60 (50)

[CALCIUM], Mg/L

RANA PIPIENS

pH	1	10	20	40	80
5.5	100	100	90	100	100
4.5	10	100	100	100	100
4.0	0	0	0	0	10

[CALCIUM], Mg/L

BUFO WOODHOUSEI

pH	1	80
5.2	100	100
4.5	100	100
4.0	0	100

[CALCIUM], Mg/L

Figure 2. Percent hatching of embryos of Bufo americanus (n=20), Bufo woodhousei (n=10), and Rana pipiens (n=10) exposed to various pH's and concentrations of calcium. Numbers in parentheses indicate the percent of hatchlings which were deformed (no parentheses = no deformities).

AMBYSTOMA JEFFERSONIANUM

pH	1	10	20	40	80
5.00	95	100	95	100	100
4.75	100	100	100	90	90 (20)
4.50	90	70	95	100	65 (30)
4.25	5	5	45	20 (25)	15 (100)
4.00	0	0	0	0	0

[MAGNESIUM], Mg/L

RANA SYLVATICA

pH	1	10	20	40	80
5.00	100	100	100	80	95 (21)
4.50	100	80	70	80 (10)	30
4.25	55	55	40	15	0
4.00	0	0	0	0	0

[MAGNESIUM], Mg/L

Figure 3. Percent hatching of embryos of Ambystoma jeffersonianum and Rana sylvatica (n=20) exposed to various pH's and concentrations of magnesium. Numbers in parentheses indicate percent of hatchlings which were deformed (no parentheses = no deformities).

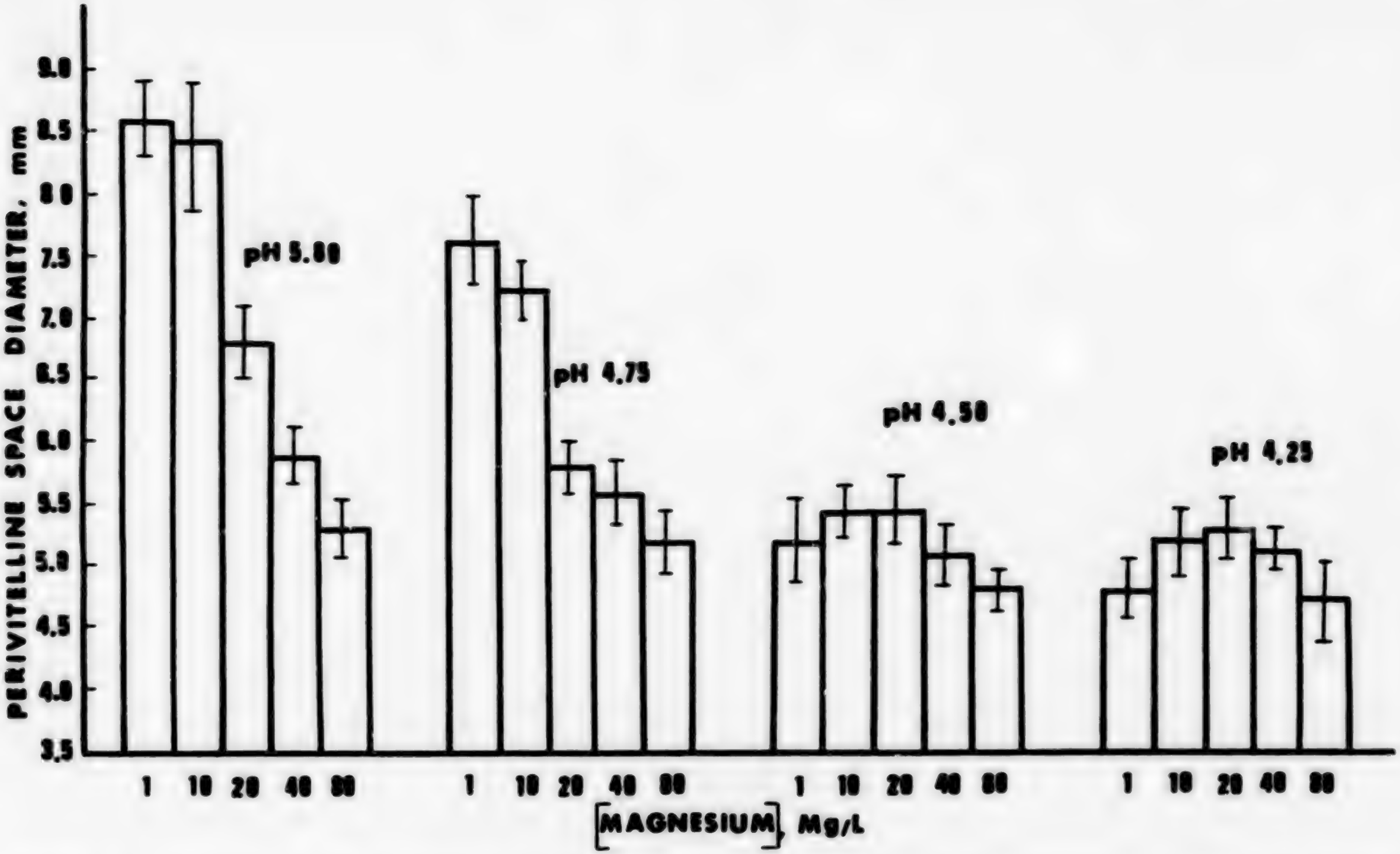

Figure 4. Diameter of the perivitelline space in embryos of *Ambystoma jeffersonianum* exposed to various pH's and concentrations of magnesium. Values listed are mean (± SD).

perivitelline space was reduced significantly by low pH alone, as well as by increasing concentrations of Mg. Although embryos at pH 5.8 and 4.75 with 40 and 80 mg/L Mg were curled into perfect spheres, they still hatched. However increasing numbers remained trapped and died as the pH declined. The numbers of hatchlings with bent spines (probably a direct result of curling) also significantly increased at low pH's and high concentrations of Mg.

Rana sylvatica - Increased magnesium concentration at pH's 4.25 and 4.50, but not at pH 5.8, significantly diminished the frequency of hatching (Figure 3). Magnesium had no effect on early mortality of embryos of R. sylvatica. At pH 4.00, no embryos reached neurulation, whereas 85 to 100% survived until hatching at pH 4.25 and 4.50 (at all Mg concentrations). The degree of curling was directly proportional to the concentration of Mg at all pH's, with embryos constricted into tight spheres at 40 and 80 mg/l. Thus the difference in percent hatching between pH 4.25 and pH's 4.50 and 5.80 at 40 and 80 mg/L Mg was attributable to the ability of embryos to escape from the vitelline membrane, and not to the degree of constriction.

2.3.3 Effects of Sodium

Ambystoma jeffersonianum - An increase in the concentration of Na increased ($\alpha = 0.10$) hatching of A. jeffersonianum embryos at pH 4.25, although much less so than Ca or Mg did, and for different reasons (Figure 5). The major cause of death at pH 4.25 and 4.50 was membrane constriction. The small increase in hatching at pH 4.25 with 80 mg/L Na was due to a lessened severity of curling. At pH 4.00, 85 to 90% died before neurulation in 1 to 20 mg/L Na; 50 to 60% survived at 40 and 80 mg/L but were later killed by membrane constriction. Increasing concentrations of Na significantly reduced the diameter of the perivitelline space at pH 5.80, 4.75 and 4.50 (Figure 6). The significant increase in diameter at pH 4.25, 80 mg/L Na, was the only incidence of reduced curling at a high concentration of any ion.

Rana sylvatica - The concentration of Na had no effect on the number of early deaths at each pH and cessation of development accounted for all mortality at pH 4.00 (Figure 5). An increase in the concentration of Na at pH 4.25 caused embryos to become progressively more curled, which resulted in significantly higher mortality. Embryos at pH 4.5 (40 to 80 mg/L Na) were also very curled, but were able to hatch. Curling was less severe at pH 5.8 and had no effect on hatching.

2.4 DISCUSSION

Amphibian embryos placed at low pH's were killed by two distinct processes. At extremely low pH's, embryos stopped development soon after exposure to test solutions. At more moderate but still lethal pH's, embryos became curled within the vitelline membrane and failed to hatch. The addition of Ca, Mg, or Na modified these responses to varying degrees, depending on the ion and the species being tested. For example, Ca prevented early mortality of embryos in Ambystoma, B. woodhousei, and R. pipiens at low pH's (< 4.5). Calcium induced the curling defect in embryos of R. sylvatica at all pH's, but did not cause curling in embryos of B. americanus. Magnesium was less

AMBYSTOMA JEFFERSONIANUM

pH	1	10	20	40	80
5.00	100	100	90	85	100
4.75	100	80	100	100	95
4.50	30 (17)	70	35 (29)	5	45
4.25	0	0	0	5	15
4.00	0	0	0	0	0

[SODIUM], Mg/L

RANA SYLVATICA

pH	1	10	20	40	80
5.00	95	100	100 (5)	100	95
4.50	100	95	95	100	95
4.25	100 (20)	80 (19)	80	40	35
4.00	0	0	0	0	0

[SODIUM], Mg/L

Figure 5. Percent hatching of embryos of *Ambystoma jeffersonianum* (n=20) and *Rana sylvatica* exposed to various pH's and concentrations of sodium. Numbers in parentheses indicate percent of hatchlings which were deformed (no parentheses = no deformities).

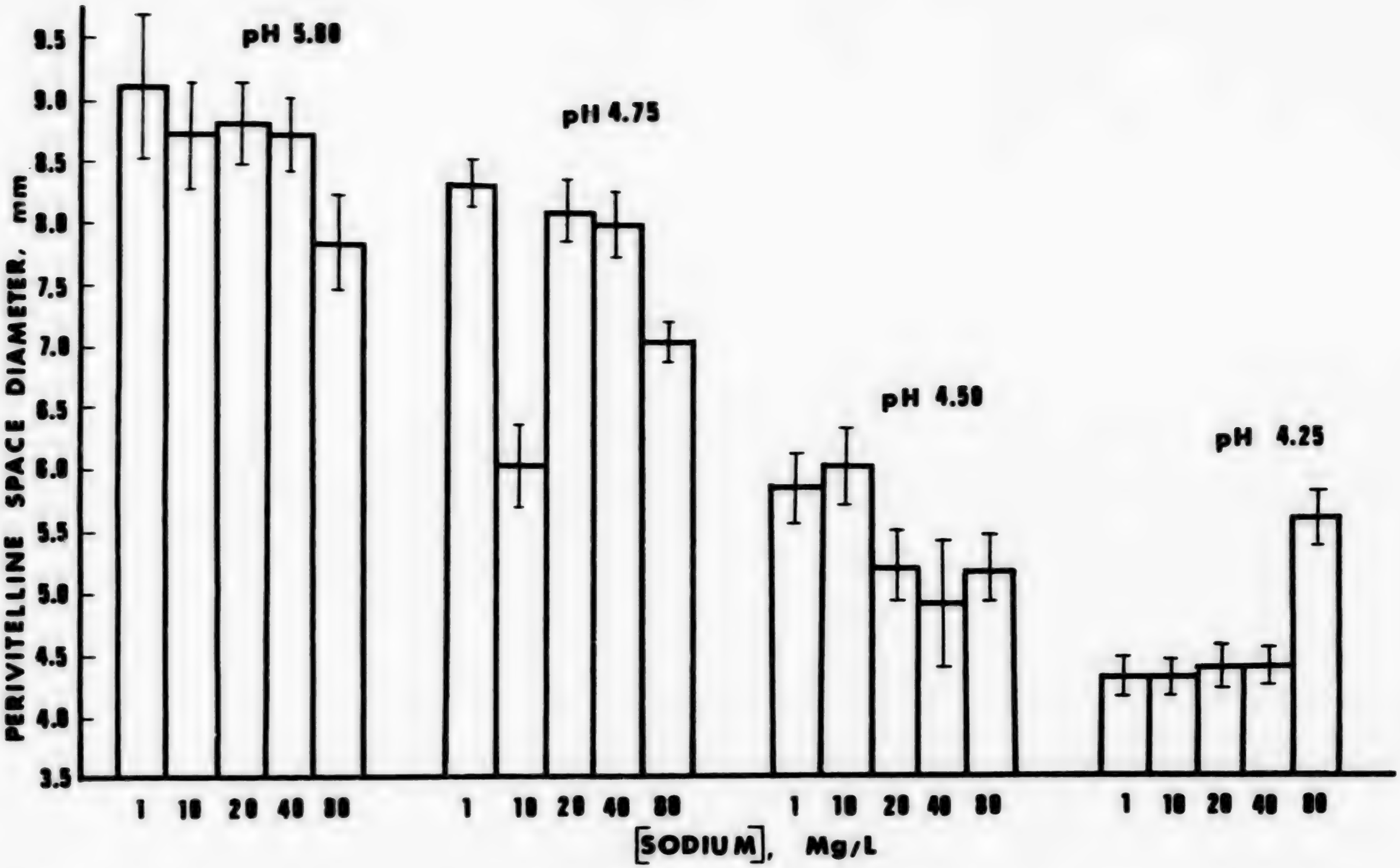

Figure 6. Diameter of the perivitelline space of embryos of *Ambystoma jeffersonianum* exposed to various pH's and concentrations of sodium. Values shown are mean (± SD).

effective at preventing early mortality, and stimulated curling in embryos of *A. jeffersonianum* and *R. sylvatica* at all pH's. Sodium had only a slight effect on early mortality and moderately increased the curling defect in these 2 species. Embryos of *Ambystoma* salamanders with the curling defect could generally hatch, while those of *R. sylvatica* remained trapped and died. Since early mortality of both species was reduced by ion additions, this differential ability to hatch when curled is the reason why more embryos of *Ambystoma*, and fewer *R. sylvatica*, hatched at low pH's and high ion concentrations. The combined influence of low pH and high concentrations of ions produced tighter curling and higher mortality of *R. sylvatica*, than when either variable was tested separately. For example, embryos of *R. sylvatica* could hatch at pH 5.8 and 80 mg/L Ca or Mg, yet mortality increased sharply at pH 4.5 with the same ion concentration. Embryos at this same pH with lower concentrations of ions were less curled and could hatch. The degree of curling in embryos of *A. jeffersonianum* responded in a similar manner to pH and Mg concentration (< 40 mg/L). However, the effect of low pH could not be expressed at 80 mg/L, since the perivitelline space had already reached a minimum size. Even at pH 5.8 (80 mg/L Mg), embryos of *A. jeffersonianum* underwent considerable curling; further declines in pH only increased this effect slightly. Salthe (1965) also reported that low pH and high concentrations of cations acted synergistically to reduce the volume of the perivitelline space.

Increasing the concentration of a specific cation from 1 to 10 mg/L did not lower low pH-induced early mortality or influence final hatching in most experiments, but did reduce the diameter of the perivitelline space. In contrast, comparing the results of the experiments which varied Mg and Na reveals that hatching of embryos of *A. jeffersonianum* and *R. sylvatica* was influenced by just 1 mg/L Mg. The 1 mg/L Na test solution and 1 mg/L Mg test solution were identical except that the former did not contain any Mg. The addition of only 1 mg/L Mg caused hatching of embryos of *A. jeffersonianum* to increase from 30 to 90% (at pH 4.50) and lowered the hatching of *R. sylvatica* from 100 to 55% (at pH 4.25). Both species were more curled in the Mg solution at these pH's and all mortality in Na and Mg test solutions was due to the curling defect. However *A. jeffersonianum* was better able to escape when Mg was present even though they were more curled; *R. sylvatica* was not and suffered higher mortality. These results are consistent with the differential effects of high concentrations of Mg (20 to 80 mg/L) on hatching of each species in acidic water. These observations on the importance of small quantities of Mg are probably too limited in scope for definitive conclusions to be drawn. However the results raise an interesting point regarding the possible interaction between cations. Perhaps it is not the absolute concentrations of cations that are most significant, but their relative levels or ratios. McWilliams (1983) has found this to be the case in his studies of survival of trout in acidic water.

The reduction in early mortality by high concentrations of Ca may be due to the well known ability of this cation to reduce membrane permeability. Calcium may prevent or slow the penetration of hydrogen ions into the perivitelline space, or into the embryo. We demonstrate in Chapter 3 that low pH kills tadpoles by disruption of ionic balance. Influx of sodium is inhibited and efflux is greatly accelerated, causing massive losses of Na and subsequent death. Increasing the concentration of Ca in water lowered the sensitivity of tadpoles to low pH by reducing the lethal sodium efflux. The disruption in ion balance of fish embryos at low pH has also been reported (Eddy and Talbot, 1985). We have observed a similar disruption of Na balance in post-tailbud embryos exposed to low pH (Freda and Dunson, unpub. obs.). If this phenomenon also occurs in young embryos, the reduction in Na efflux by Ca may be the reason for its protective effects.

The constriction of embryos by the vitelline membrane was the primary cause of death because they do not become curled and survive if the vitelline membrane is removed (Dunson and Connell, 1982). The most likely explanation for an increase in the curling defect with increasing concentration of ions is deactivation of the hatching enzyme. *In vitro* studies on the hatching enzyme of fish and amphibians have demonstrated a strong inhibitory effect of low pH, and increases in Ca, Mg, and Na to a lesser extent (Yamagami, 1973; Katagiri, 1975; Urch and Hedrich, 1981). The hatching enzyme of amphibian embryos is responsible for changes in the vitelline membrane, which allow it to expand in response to the osmotic uptake of water, enlarging the perivitelline space. The digestive action of the enzyme also facilitates the rupture of the vitelline membrane by muscular contractions of the larvae at hatching. The more severe curling of embryos, when high concentrations of both hydrogen ions and divalent cations were present, may be the result of additive inhibition of hatching enzyme activity. The hatching enzyme is synthesized and released from glands on the forehead of embryos; this explains why a small hole appeared near the head of curled embryos, allowing their escape (Carroll and Hedrick, 1974; Yoshizaki and Katagiri, 1975).

As in previous studies, embryos of *A. jeffersonianum* were found to be considerably more sensitive to low pH than those of *R. sylvatica* (Tome and Pough, 1982; Pierce *et al.*, 1984). Embryos used in this study were much more sensitive to low pH than those used in these two previous studies. We observed no hatching of *A. jeffersonianum*, *A. maculatum*, or *R. sylvatica* in ASW (without ion additions) at pH 4.00. Pough and Wilson (1977) reported 45 and 58% hatching of *A. maculatum* (at 15 C) and *A. jeffersonianum* (at 5 C), respectively, at pH 4.00. Pierce *et al.* (1984) and Tome and Pough (1982) reported no increase in mortality of embryos of *R. sylvatica* at pH 4.00. However Dale *et al.* (1985) reported very high mortality in embryos of *R. sylvatica* and *A. maculatum* at pH 4.00. Such differences in lethal limits might be explained by geographic variation in tolerance to low pH and/or differences in experimental protocol. Test solutions used by other investigators were more concentrated than the ASW used in our bioassays. Experimental solutions used by Pough and Wilson (1977) and Tome and Pough (1982) contained 44 mg Na/L and less than 1 mg K, Ca, and Mg/L. Experiments of Pierce *et al.* (1984) were conducted in water containing 13 mg Na/L, 1 mg K/L, 7 mg Ca/L, and 6 mg Mg/L. Water used by Dale *et al.* (1984) contained 3 mg Na/L, 13 mg Ca/L, and less than 1 mg K and Mg/L. While the solutions used in these studies contained higher concentrations of cations than our ASW, this fact probably does not account for the observed differences in frequency of hatching, unless increasing the concentration of more than one ion has a synergistic effect. Our results indicate that increases in cation concentration decrease rather than increase hatching in embryos of *R. sylvatica*. Percent hatching of *Ambystoma* reported by Pough and Wilson (1977) also exceeds our values for embryos tested at high cation concentrations (80 mg/L Ca or Mg).

In acidic (pH < 5.0) breeding ponds, the concentrations of ions (Na, Mg, Ca) may influence the hatching of amphibian embryos. Effects are likely to be difficult to detect because ion levels are usually very low (< 2 mg/L) in acidic ponds, and only exceed 10 mg/L in ponds of high pH where mortality does not occur (Chapter 4). The present demonstration that several cations do influence low pH tolerance of amphibian embryos points out the need for detailed chemical descriptions of laboratory solutions and ponds used in toxicity tests. While our study does not confirm or refute geographic variation in tolerance of embryos to low pH, it does indicate that a great deal of caution is also necessary in interpreting the results of studies done in media differing in cation composition.

3.0 PHYSIOLOGICAL EFFECTS OF LOW PH WATER ON ION BALANCE IN AMPHIBIAN LARVAE.

3.1 INTRODUCTION

Most research on the physiological effects of low pH on aquatic organisms has been done on fish. Packer and Dunson (1970, 1972) were the first to describe disruption of ionic-regulatory processes in fish after low pH exposure. Lowering the pH of the external medium depressed sodium influx and markedly increased sodium efflux, culminating in death from lowered body sodium content. Several recent studies have confirmed that ionic-regulatory failure is a major cause of death in fish at low pH (McDonald *et al*., 1980; McWilliams, 1980). Increasing the external calcium concentration slows sodium efflux, thus extending survival time or preventing mortality (Brown, 1981). Fish maintained in water high in calcium also exhibited faster recovery of ionic regulatory mechanisms after low pH exposure (McDonald *et al*., 1983). McDonald *et al*. (1980) found that calcium levels dictated the type of low pH response observed. Trout tested in hard water suffered a blood acidosis and moderate depression of plasma sodium and chloride levels, while fish in soft water experienced a massive loss of sodium and chloride with no change in plasma pH. McWilliams (1983) reported that low pH removed calcium ions from the surface of the gill membrane and that acid tolerant strains lost calcium more slowly.

The purpose of the present study was to examine disruption of ionic balance in amphibian larvae exposed to low pH water. Our three main objectives were: (1) To determine the cause of death during acute exposure to lethal pH's, (2) To describe compensatory adjustments made by tadpoles during chronic sublethal exposure to low pH, and (3) To determine whether tadpoles in naturally acidic ponds were affected physiologically by low pH.

3.2 METHODS

3.2.1 *Acute Exposure*

Experimental Animals - Four species of amphibians were studied. Leopard frog (*Rana pipiens*) tadpoles were obtained through laboratory matings (Rugh, 1952) of Wisconsin frogs purchased commercially. Bullfrog (*R. catesbeiana*) tadpoles were purchased locally (November 30, 1982) and were used 5 to 20 days later. Green frog (*R. clamitans*) tadpoles were captured (October 16, 1982) in a small forest pond in Centre Co., PA, and held in the laboratory until experimentation (10-20 days). On March 1, 1982, marbled salamander larvae (*Ambystoma opacum*) were taken from a temporary forest pond in Passaic Co., N.J., and held until their use 18 days later. All animals were maintained in dechlorinated State College tap water (SCW, Table 4) at room temperature (20-22 C). Tadpoles were fed Nasco frog brittle while salamander larvae were offered live larval *Artemia* *ad lib* . Tadpoles were staged according to Gosner (1960).

Dry mass was determined by weighing tadpoles (±0.0002 g) after 12 h at 95 C. Dried animals were digested in concentrated nitric acid and the solution was diluted with distilled water. Sodium and potassium were then

Table 4. pH and ion concentrations of State College tap water (SCW), artificial soft water (ASW), and Bear Meadows Bog water (BOG).

Water	pH	Na	K	mg/L Ca	Mg	Cl
SCW	7.45	3.9	0.4	27.8	17.5	12.3
ASW	5.80	1.0	1.0	1.0	0	4.2
BOG	4.25-4.50	0.6	0.7	2.0	0.3	0.2

analyzed with a Varian Techtron model 1280 atomic absorption spectrophotometer. Unless otherwise noted, all animals were acclimated and unfed for three days (10 animals per 5 L) in artificial soft water solution (ASW, Table 4) prior to all experiments.

Sodium Influx - Sodium influx was studied by using ^{24}Na labeled NaCl solutions prepared by neutron activation at the Nuclear Reactor Facility of The Pennsylvania State University. Individual tadpoles were added to 1 L of NaCl free ASW to which 50 μL of 1 M ^{24}NaCl (750 μCi/ml) had been added. Bath pH was adjusted with dilute H_2SO_4 or NH_4OH (used only for pH 6.5 and 7.0 baths) and generally did not change by more than 0.05 units during the course of an experiment. After 30 min loading periods, larvae were placed for 1 min in an ASW rinse having the same pH as the test solution. Animals were then placed alive into a test tube and counted in a NaI crystal connected to a Canberra series 30 Multichannel analyzer. Unless otherwise noted, all larvae received 4 consecutive 30 min loading periods. Animals that died before the termination of the experiment were removed, rinsed, and counted shortly after death. Death was defined as the lack of response to mechanical stimuli. Influx was calculated according to Stokes and Dunson (1982). At the end of each experiment, body sodium content of each animal was determined. All procedures were carried out at 20-22 C.

Sodium Efflux - Sodium efflux from larval amphibians was measured by loading them in NaCl free ASW (10 per 5 L) to which 0.5 ml of the 1 M ^{24}NaCl had been added. After a 12 h loading period, the tadpoles were kept in 5 L of unlabeled ASW for 30 min as a rinse. Tadpoles were then counted and placed in unlabeled 1 L ASW baths of varying pH. Larvae were subsequently counted 4 times after consecutive 30 min efflux periods. Other aspects of this procedure are identical to those described for influx.

Urodele Tolerance - The relationship between environmental pH, sodium efflux, and survival time was investigated in *A. opacum* larvae. After an initial loading period, 4 larvae each were placed in baths having pH's of 3.0, 3.5 and 4.0. Efflux measurements were made every 1.5 h until death, and survival times were noted. Animals were held in SCW previous to this experiment.

Anuran Interspecific Differences - Sodium influx and efflux of *R. pipiens*, *R. clamitans*, and *R. catesbeiana* were measured during acute exposures to pH's ranging from 2.5 to 7.0. This series of experiments was performed to determine whether acute exposure to low pH affects sodium balance and, if so, to see if tolerances among species are related to the magnitude of disruption. Tolerance was documented by measuring the survival times of 10 individuals of each species placed in pH 3.0 ASW.

Calcium Effects - Sodium influx and efflux in *R. pipiens* acutely exposed to pH 3.5 were measured in ASW solutions having 0.025 or 5 mM calcium (added as $CaCl_2$). One continuous 2 h influx or efflux period was used rather than the usual 4, 30 min periods in order to test for possible handling effects.

Dark Water Effects - Laboratory sodium flux determinations were also made on tadpoles in bog water with high concentrations of organic compounds relative to ASW solutions of similar pH. The bog water was obtained from Bear Meadows (Saber and Dunson, 1978). Dissolved ions in the bog water occur in low concentrations (Table 4). Organic acids in the water were measured with a Hach Tannin/Lignin test kit (expressed as mg/L tannic acid). The bog water contained 2.8 mg/L tannic acid. Sodium influx was measured in individual *R. pipiens* tadpoles placed in 1 L of ^{23}NaCl free ASW containing 50 μL of 1 M ^{24}NaCl (1000 μCi/ml). Twenty-five L of the ^{24}NaCl were added to 1 L of bog water (26 μM Na) so both the bog water and ASW solutions had approximately the same final sodium concentration (50 μM). The ASW solutions were adjusted with dilute H_2SO_4 to the same pH (4.50) as bog water. After a 15 min loading period, animals were kept for 1 min in an ASW rinse (pH 4.50) and then placed alive into a test tube and radioassayed (two replicates). Sodium efflux from *R. catesbeiana* tadpoles was measured after loading them for 4 h in NaCl free ASW containing 50 μL of 1 M ^{24}NaCl. After 30 min in unlabeled ASW as a rinse, tadpoles were then counted and placed in unlabeled, 1 L ASW and bog water baths. Tadpoles were recounted after 1 h.

3.2.2 Chronic Exposure

Sodium Fluxes - Sodium balance during chronic exposure to low pH water was studied by measuring initial and final body ion contents and sodium fluxes. *R. pipiens* and *R. catesbeiana* were maintained in pH 4.0 ASW for approximately 2 and 7 days respectively, with *R. catesbeiana* receiving an additional treatment at pH 3.5 for 1 day. Control treatments in pH 5.8 ASW were also performed. After exposure periods, surviving tadpoles were analyzed for both sodium and potassium. To test for possible changes in sodium fluxes during chronic exposure, sodium influx and efflux in *R. pipiens* were measured at pH 4.5 after 3 and 7 days acclimation to this pH. Tadpoles were kept at a density of 5 animals per 5 L and were fasted during all chronic acclimations. During 7 day treatments, solutions were changed at 3.5 days.

Statistical Tests - All results are reported as means ±SD. Significant differences between means were determined with a Mann-Whitney U test at a significance level of $P < 0.05$. Regression lines calculated with a Hewlett Packard model 85 computer were compared using a modified F test ($P < 0.05$; Neter and Wasserman, 1974).

3.2.3 Field Measurements

Study Ponds - The 2 temporary forest ponds used are approximately 2.6 km apart within Rothrock State Forest, situated in the Ridge and Valley province of Central Pennsylvania. The vegetation is dominated by oak-hickory (*Quercus-Carya*), with an understory of rhododendron (*Rhododendron*), mountain laurel (*Kalmia*), and blueberry (*Vaccinium*). McAlevy pond is very acidic (pH 4.05-4.90) while Barrville pond is higher in pH (pH 5.74-6.37); both are very dilute in solutes (Fig. 10) and darkly stained (mean tannic acid concentrations were 2.2 and 2.4, respectively). The source(s) of acidity in McAlevy (subsequently designated pond L) have not been determined, but humic acids, *Sphagnum* moss, and acid rain are likely candidates. The reason for Barrville's higher pH is not known. In addition to wood frog tadpoles, Barrville pond (subsequently designated pond H) supports large numbers of adult newts (*Notopthalmus v. viridescens*), spotted salamander larvae (*Ambystoma maculatum*), and Jefferson salamander larvae (*A. jeffersonianum*). Only 1 *A. maculatum* larva was found in pond L.

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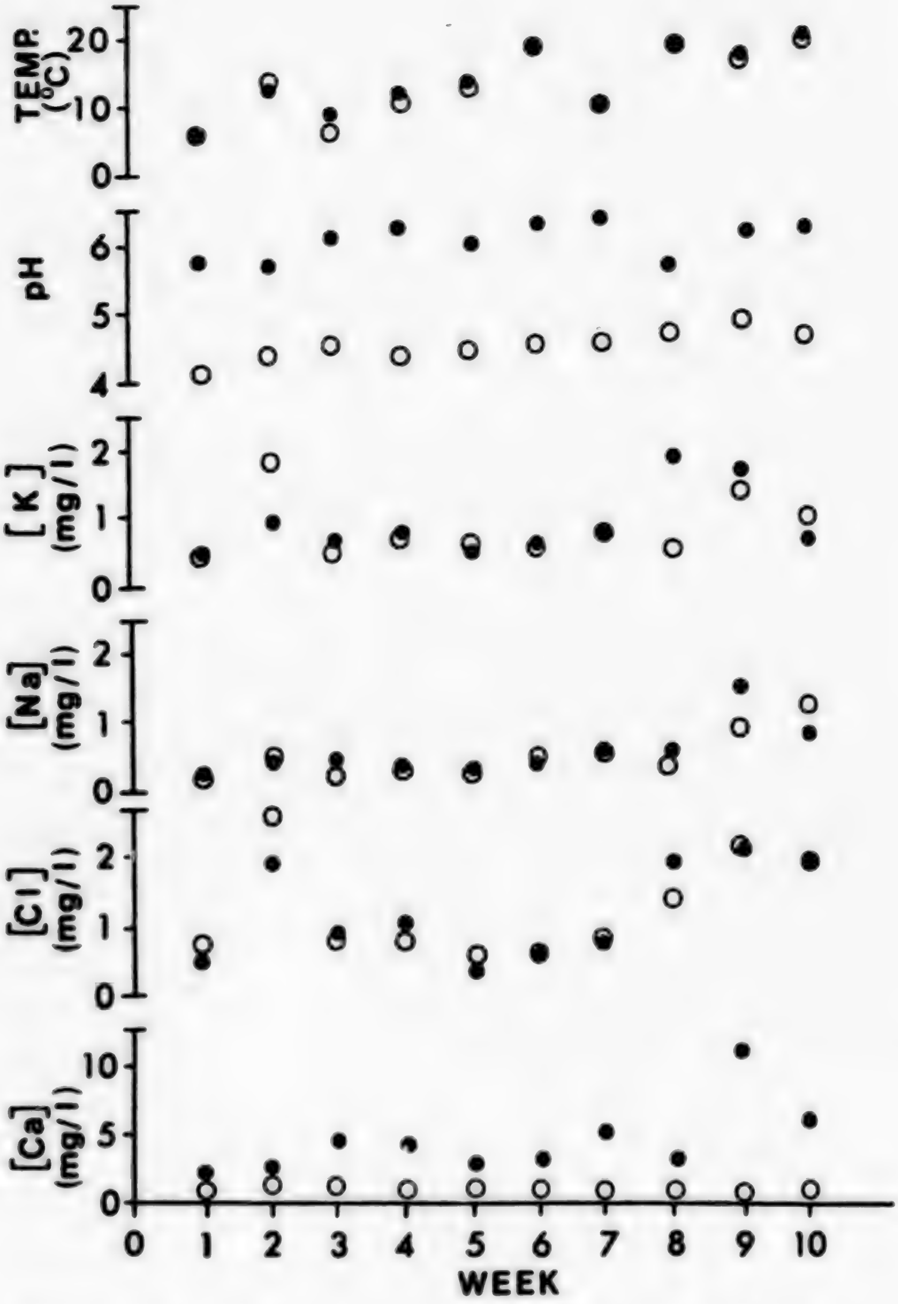

Figure 10. Ion concentrations (Na, K, Ca, Cl), pH, and temperature of both pond H (●) and pond L (O) during the study period.

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Pond water chemistry determinations were made once weekly, simultaneously with tadpole collections. Water samples were collected 5 cm below the surface, at the center of each pond. pH's were taken in both the field and laboratory and did not change by more than 0.05 units after transport. Since our laboratory pH meter (Beckman model 4500) had both higher precision and accuracy than the Leeds and Northrup field instrument, laboratory pH values are reported hereafter. Temperature was measured at the site of water collection with a standard mercury thermometer. Water samples were collected in 500 ml polyethylene bottles, placed in an ice filled cooler while in transport, and stored at 4 C until analysed. pH was measured within 24 h of collection. Samples were filtered through a 0.45 μm Millipore filter; cation (Na, K, Ca) concentrations were determined by atomic absorption spectrophotometry and chloride by mercuric nitrate titration within 36 h (APHA et al., 1975).

Body Water and Ion Concentration - R. sylvatica tadpoles were collected by dip netting once a week from hatching until metamorphosis. They were collected at the same time as tadpoles used for body analysis. Approximately 50 captured tadpoles in 20 L of their native pond water were returned to the laboratory. All animals were analyzed within 12 h and kept at temperatures approximating pond conditions until that time. Wet mass, dry mass, and percent water were determined by weighing towel dried tadpoles (n = 10 for each pond), with a subsequent reweighing after 12 h at 95 C. Subtraction of dry mass from wet mass provided estimates of percent water. Body sodium and potassium concentration were obtained by digesting the dried tadpoles in concentrated nitric acid, followed by dilution, and analysis with a Varian Techtron model 1280 atomic absorption spectrophotometer. Body chloride concentration was determined by mercuric nitrate titration of wet tadpoles homogenized in a Thomas teflon pestle tissue grinder. The mean percent water of tadpoles from each collection date was used to transform Cl values from a wet mass to a dry mass basis.

Low pH Tolerance - To evaluate changes in pH sensitivity during development, survival times of 10 tadpoles from each pond were measured in 1 L of artificial soft water solution (ASW, Table 4) adjusted to pH 3.00 with dilute sulfuric acid. pH 3.0 was selected as a test pH since tadpoles were capable of survival at pH 3.5. Exposure to pH 3.25 resulted in survival times exceeding 24 h, which caused problems with upward pH shifts and buildup of metabolic by-products because of the high density of animals in this particular experiment. Experiments were conducted at 22-25 C. Death was defined as the lack of response to prodding. Tadpoles killed in survival time experiments from weeks 3 through 9 were analyzed for sodium and potassium concentrations while body chloride concentrations were only determined during week 9. For comparison, the effect of age on pH tolerance was also investigated using R. pipiens tadpoles. Once a week from hatching through 8 weeks of age, 10 larvae were removed directly from tap water and placed in 1 L of ASW at pH 3.0

Sodium Efflux - Unidirectional sodium effluxes of tadpoles from ponds H and L were measured in their own as well as in each other's native pond with the use of a radioactive tracer. Tadpoles were captured during week 4, returned to the laboratory and placed in radioactively labeled (^{24}Na) ASW (pH 5.8) for 12 h. Both pond H and L tadpoles were exposed to identical pH's during this loading period to insure equal levels of sodium uptake. Tadpoles were then transferred to unlabeled ASW (pH 5.8) for 2 h as a rinse. Isotopic activity of each tadpole was measured with a NaI crystal connected to a Canberra series 30 multichannel analyzer. Next, 10 tadpoles were placed in each of 4 14 cm x 18 cm x 7 cm nylon screen cages and transported to the ponds in pH 5.8 ASW. Each pond received 1 group of L and H tadpoles. The tadpoles were kept in the ponds for approximately 20 h, then returned to the laboratory in the pond water of their specific treatment for recounting. Since individual tadpoles were not marked, only the pooled mean counts per minute (CPM) of the 10 tadpoles in each treatment was obtained. Percent loss of sodium was estimated by comparing initial and final mean CPM per tadpole in each treatment (corrected to 24 h).

All data are reported as means ±1 standard deviation. A two tailed student's t-test was used to detect significant differences ($\alpha = 0.05$) in physiological variables between groups of tadpoles (on a weekly basis). Regression lines were compared using a modified F test (Neter and Wasserman, 1974) at a 0.05 significance level.

3.3 RESULTS

3.3.1 Acute Exposure

The relationship between sodium efflux, pH, and survival time in *A. opacum* is illustrated in Figure 7. As pH declines, sodium efflux increases, and survival time decreases. Individual differences in survival time at a specific pH are also reflected in the magnitude of the sodium efflux at pH 3.5 and 4.0.

Sodium influx and efflux of 3 different species of tadpoles are presented in Figures 8 and 9. Influx of sodium was depressed linearly and significantly to near zero by decreasing pH. Statistical regressions of influx against pH were performed on values obtained at and above the highest pH to produce near zero influxes. Although the large sodium efflux experienced at the lowest pH's could have reduced influx measurements, we believe this is not a major factor since influx decreased linearly throughout the upper part of the pH range tested (4.0-7.0) while efflux rose sharply only below 4.0. Influx regression lines for *R. catesbeiana* and *R. pipiens* are not significantly different, while both of these are significantly lower than that for *R. clamitans*. As pH declined, sodium efflux in all 3 species increased, although to a varying extent (*R. pipiens* > *R. catesbeiana* > *R. clamitans*). Regression lines of efflux against pH differed significantly among the three species. Percent reduction in body sodium content of animals killed during the course of the flux experiments is reported in Table 5. Our isotopic efflux measurements were 5 to 8% higher than net flux estimates made from survival time and the net amount of sodium lost in tadpoles that died during flux experiments.

The effects of low pH on sodium fluxes of the 3 species are related directly to their sensitivity to low pH and to control body sodium contents (Table 5). Differences in tolerance are even more evident in chronic exposures. *R. pipiens* could not withstand exposure to pH 4.0 while *R. catesbeiana* could. Additionally, *R. catesbeiana* could not survive in pH 3.5 water, while *R. clamitans* could live for several weeks at this pH.

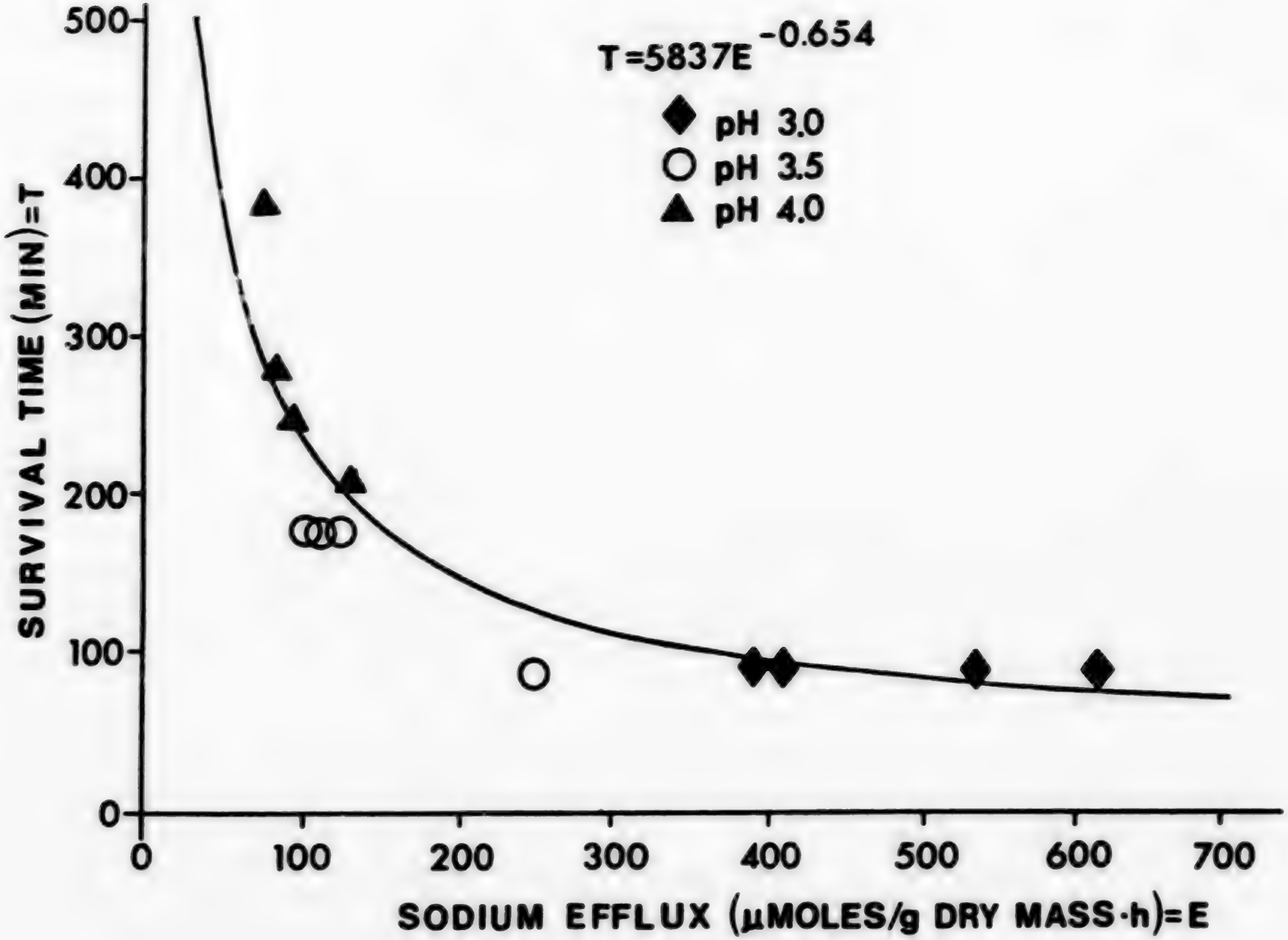

Figure 7. The relationship between sodium efflux and survival time of salamander larvae (A. opacum) placed in various pH solutions. $r^2 = 0.86$, $P < 0.001$.

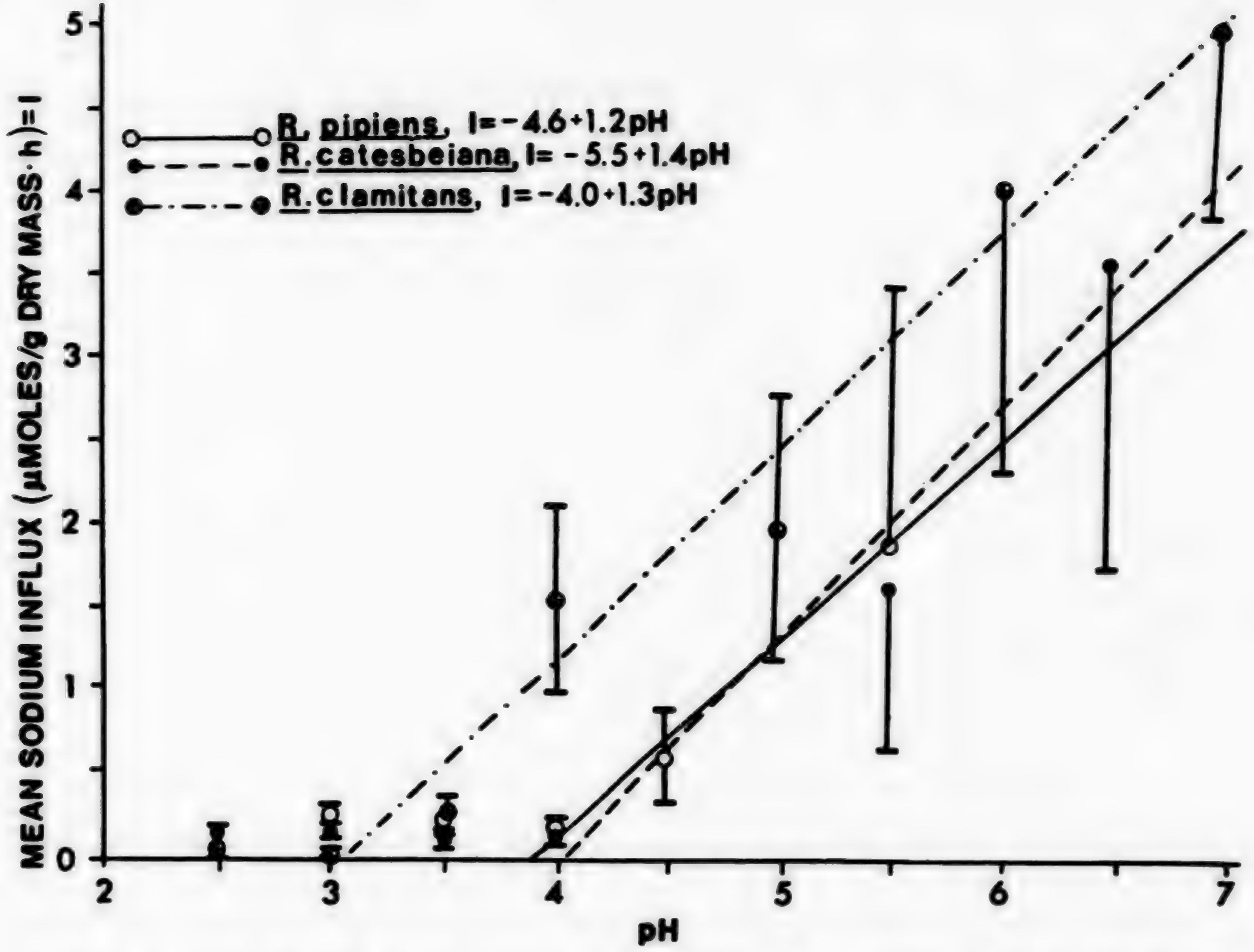

Figure 8. Mean sodium influx (±SD) in three species of anuran tadpoles during the first two hours of exposure to various pH's (n = 5 for each species at a specific pH. R. pipiens (r^2 = 0.45, P < 0.10). R. catesbeiana (r^2 = 0.63, P < 0.05). R. clamitans (r^2 = 0.76, P < 0.001).

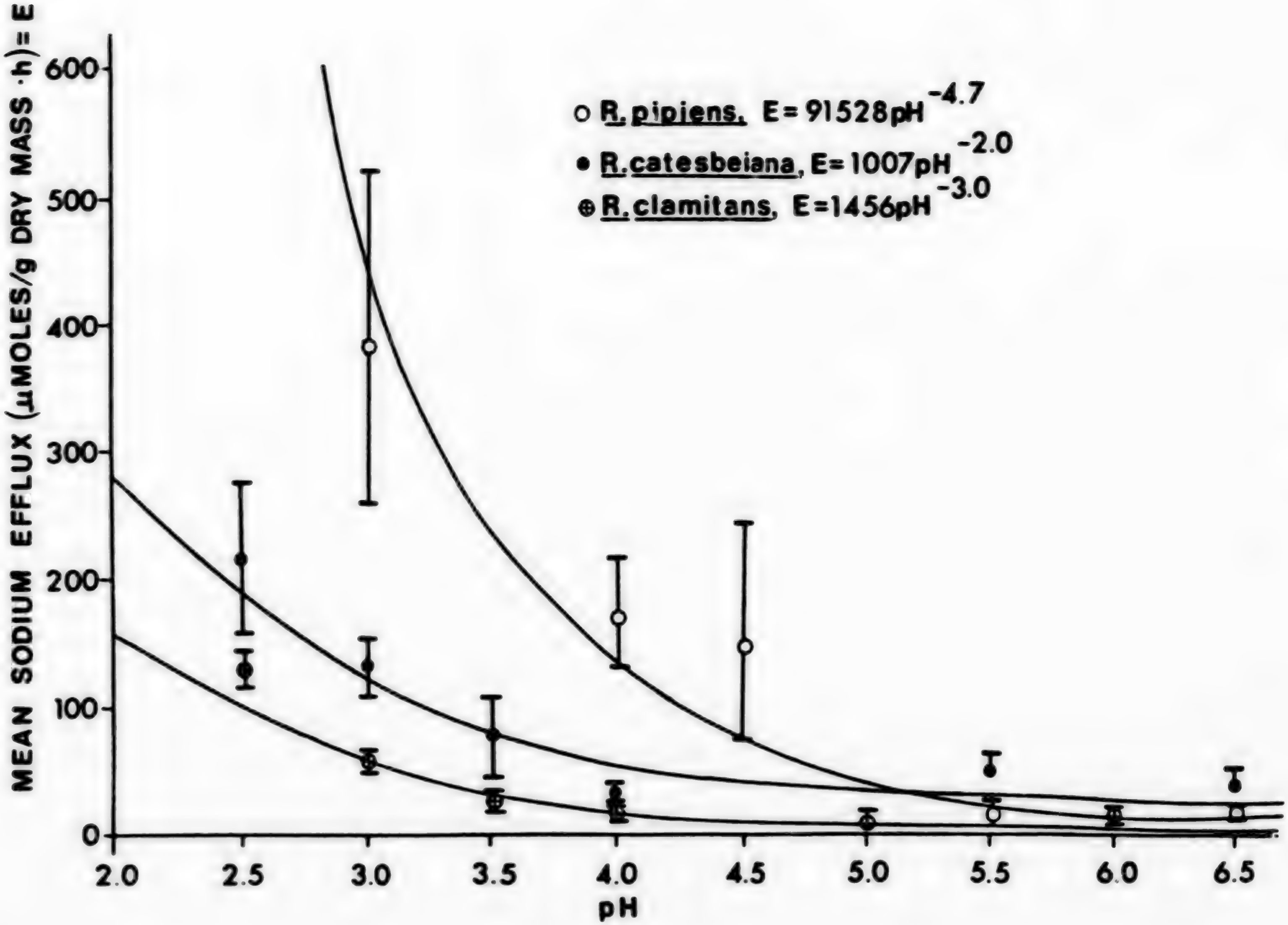

Figure 9. Mean sodium efflux (±SD) in three species of anuran tadpoles during the first two hours of exposure to various pH's (n = 5 for each species at a specific pH). R. pipiens ($r^2 = 0.84$, $P < 0.001$). R. catesbeiana ($r^2 = 0.59$, $P < 0.001$). R. clamitans ($r^2 = 0.74$, $P < 0.001$).

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Table 5. Survival time, initial body Na content, and percent reduction in body sodium content in three species of anuran tadpoles killed by acute exposure to pH 3.0. Values equal mean ± SD. Sample size in parentheses.

Species	g Initial dry mass	min Survival time	μmoles/g dry mass Initial body Na content	% reduction in body Na at death
R. pipiens	0.017 ± 0.009 (n=45)	70 ± 4 (n=10)	1365 ± 301 (n=10)	48%
R. catesbeiana	0.183 ± 0.053 (n=60)	271 ± 125 (n= 7)	573 ± 105 (n=20)	39%
R. clamitans	0.127 ± 0.032 (n=60)	372 ± 180 (n= 7)	369 ± 84 (n=19)	52%

The effect of environmental calcium concentration on sodium influx and efflux in *R. pipiens* during acute exposure to pH 3.5 is reported in Table 6. Sodium efflux showed a significant reduction at higher calcium concentrations, whereas influx did not change. Increasing calcium from 0.025 to 5 mM lengthened survival time of *R. pipiens* tadpoles (n = 10) at pH 3.5 by 4-fold.

Neither sodium influx nor efflux were significantly different when measured in bog water or ASW having the same pH. *R. pipiens* tadpoles (3.6 ± 1.1 mg dry mass) took up sodium at a rate of 2.4 ± 0.9 (n = 3) and 2.7 ± 0.9 (n = 3) moles/g dry mass • h in pH 4.5 bog water and ASW respectively. *R. catesbeiana* tadpoles (94.1 ± 27.9 mg dry mass) had effluxes of 13.9 ± 8.4 (n = 4) and 20.0 ± 11.4 (n = 5) moles/g dry mass • h in pH 4.25 bog water and ASW respectively.

3.3.2 Chronic Exposure

Sodium and potassium contents of tadpoles chronically exposed to low environmental pH are presented in Table 7. After approximately 2 days in a pH 4.0 ASW solution, *R. pipiens* tadpoles were significantly lower in body sodium and unchanged ($P < 0.05$) in body potassium content. At pH 4.0, 3 animals died, while the remaining 7 animals lost 36% of their body sodium. *R. catesbeiana* tadpoles placed in pH 3.5 ASW for 0.9 days were also significantly lower in sodium and unchanged in potassium. These animals were severely stressed as evidenced by a 50% mortality and a 62% reduction in body sodium. During long term exposures to more moderate pH's, tadpoles were able to lose more than 50% of their body sodium while this would have been lethal in an acute exposure to very low pH (pH 3.0). *R. catesbeiana* tadpoles maintained chronically for three and seven days in a sublethal pH 4.0 solution still were significantly lower (21 and 23% respectively) in body sodium content, while potassium was unchanged from control values.

"Acclimation" of *R. pipiens* tadpoles to a sublethal pH of 4.5 increased influx above levels of uptake during acute exposure to both pH 4.5 and 5.5 (Table 8). Influxes were also higher than those of the more tolerant *R. catesbeiana* at pH 5.5 and *R. clamitans* at pH 5.0. Acclimation to the test pH also significantly lowered sodium efflux as compared with acute exposures.

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Table 6. The effect of calcium concentration on sodium influx and efflux of *R. pipiens* tadpoles during acute exposure to pH 3.50 (n=5). Values represent means ± SD.[a]

[mM] Calcium concentration	µmoles/g dry mass · h Mean sodium influx	µmoles/g dry mass · h Mean sodium efflux
0.025	0.44 ± 0.49	168 ± 52
5	0.37 ± 0.44	22 ± 5

[a] Stage 25, 68 days old, 0.0309 ± 0.0134 g dry mass

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Table 7. Body sodium and potassium content (mean ± SD) of anuran tadpoles chronically exposed to low environmental pH.[a]

Species	Sample size	Duration (days)	pH range	µmoles/g dry mass Na	 K
R. pipiens	5	1.73	5.50 - 5.82	1815 ± 196	507 ± 67
R. pipiens	7	1.73	3.96 - 4.01	1154 ± 329	684 ± 222
R. catesbeiana	5	0.88	5.46 - 5.95	1080 ± 182	339 ± 30
R. catesbeiana	5	0.88	3.50 - 3.62	408 ± 89	331 ± 28
R. catesbeiana	5	7.00	5.40 - 6.35	1063 ± 233	355 ± 32
R. catesbeiana	5	2.96	3.99 - 4.24	841 ± 109	345 ± 26
R. catesbeiana	5	7.00	4.00 - 4.36	817 ± 133	313 ± 32

[a] *R. pipiens* were 22 days old, stage 25, and had a mean mass of 0.0130 ± 0.0036 g dry, while *R. catesbeiana* were stage 25 and weighed 0.1585 ± 0.0521 g dry.

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Table 8. Sodium fluxes of R. *pipiens* placed in pH 4.5 solution after 0, 3 and 7 day acclimation in the test solution (sample size below). Values represent mean ± SD.[a]

Treatment	µmoles/g dry mass • h Na influx	Na efflux
0 day acclimation	0.61 ± 0.24 (5)	160 ± 85 (5)
3 day acclimation	3.39 ± 1.76 (5)	87 ± 44 (5)
7 day acclimation	6.30 ± 3.14 (4)	69 ± 43 (5)

[a] 60 days old, stage 25, 0.0076 ± 0.0053 g dry mass.

3.3.3 Field Measurements

Breeding Cycle - *R. sylvatica* adults bred in ponds H and L during 16-20, March. Collection of tadpoles and water samples began on April 25, 1 week after hatching in both ponds (week 1). Both populations were in stages 22-23 (Gosner, 1960) during week 1 and displayed stage 25 characteristics during week 2. Stage 39 was attained by pond L tadpoles during week 9 and they were newly metamorphosed froglets during week 10. Pond H tadpoles most likely metamorphosed between weeks 8 and 9. The few remaining animals collected during week 9 were extremely small compared to the previous sample and may represent tadpoles too small to metamorphose. Pond drying may have been a factor promoting earlier metamorphosis of pond H tadpoles since it was reduced to a small puddle during week 10, while pond L was still quite full.

Water chemistry - Pond water chemistry variables for the 10 week sampling period are illustrated in Figure 10. Sodium, potassium, chloride and temperature are similar for both ponds, while calcium and pH differed. The maximum pH difference between ponds was 1.77 during week 7, while the minimum was 1.04 at week 8. The mean pH difference was 1.55. Slight increases in the concentrations of pond water ions after week 7 were most likely caused by decreases in pond volume due to evaporation.

Body mass and percent water - Wet mass, dry mass, and percent water of tadpoles over the 10 week period are presented in Figure 11. Dry masses of tadpoles from ponds H and L at week 2 only increased 20% and 16% over week 1, whereas wet masses increased by 300% and 282%, respectively. Thus "growth" during this period was mainly due to uptake of water. Mean percent water values of tadpoles from pond L were significantly lower than those of pond H during 7 of 9 weeks. The regression line of body water content ($C = g$ H_2O/individual) plotted against dry mass ($M = g$) for the 9 week study period was significantly lower ($P < 0.05$) for tadpoles from pond L as compared to pond H ($C = 0.02 + 10.11\ M$, $r^2 = 0.934$, $P < 0.001$ and $C = 0.01 + 14.67\ M$, $r^2 = 0.912$, $P < 0.001$ respectively).

Body ion concentration - The relationship between body ion concentration and age is illustrated in Figure 12. Body sodium concentration in tadpoles from pond L was significantly lower than levels from pond H during 8 of 9 weeks. Pond L/pond H body sodium ratios for these 8 weeks ranged from 0.60 during week 1, to 0.87 during week 4, with a mean of 0.71. Body chloride concentration of tadpoles from pond L was significantly lower for 5 weeks, but not different for 3 weeks. No trend is evident for potassium levels since pond L was significantly higher for 3 weeks while pond H potassium levels were greater for the other 3 weeks.

Since tadpoles from pond L grew at a faster rate and sodium concentrations declined with age, differences in body sodium could be a size rather than a pH effect. However, this is clearly not the case since the regression line for body sodium content ($C = \mu$moles/individual) plotted against dry mass ($M = g$) (over 9 weeks) for tadpoles from pond L was significantly lower ($P < 0.05$) than for tadpoles from pond H ($C = 2.2 + 750.4\ M$, $r^2 = 0.944$, $P < 0.001$ and $C = 1.2 + 1096.5\ M$, $r^2 = 0.891$, $P < 0.001$, respectively). A similar comparison using potassium was not significant ($P < 0.05$) (pond L: $C = 0.8 + 353.4\ M$, $r^2 = 0.979$, $P < 0.001$; pond H: $C = 0.2 + 381.0\ M$, $r^2 = 0.915$, $P < 0.001$).

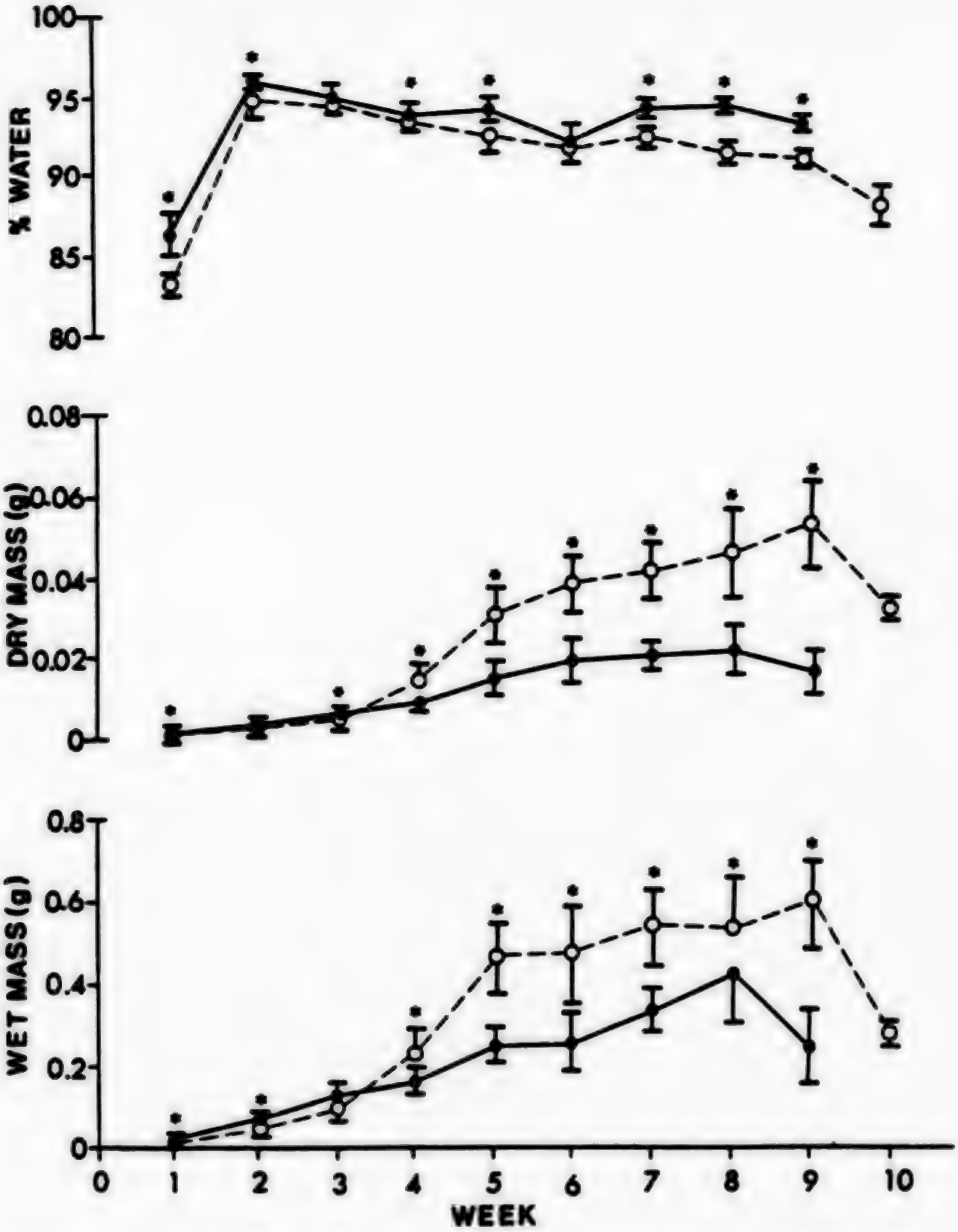

Figure 11. Mean wet mass, dry mass, and percent water content (±SD) of tadpoles from ponds H (●) and L (O) during the 10 week study period (n = 10, asterisk indicates significant difference between ponds). Lines connect the means.

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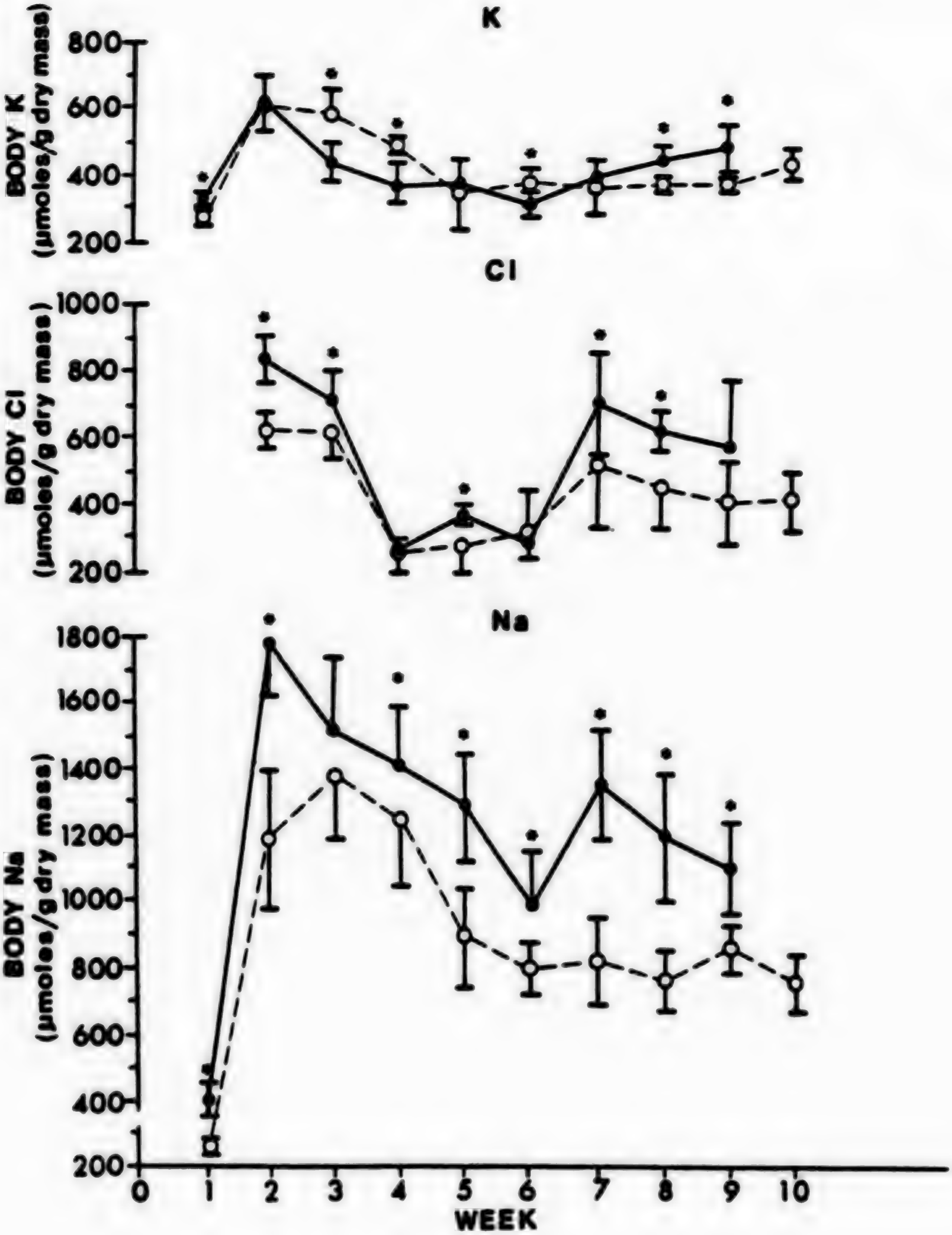

Figure 12. Body ion concentration (Na, Cl, K) of tadpoles (*R. sylvatica*) from ponds H (●) and L (O) for 10 consecutive weeks (n = 10, asterisk indicates significant differences between ponds). Values are mean ± SD. Lines connect the means.

Highly significant relationships occurred between percent water (W) and body sodium (Na = µmoles/g dry mass); (pond H: $W = 57.8\ Na^{.068}$, $r^2 = 0.923$, $P < 0.001$; pond L: $W = 56.0\ Na^{.073}$, $r^2 = 0.940$, $P < 0.001$). Regressions of potassium and chloride with percent water were not significant. The difference in mean body sodium concentration between the two ponds is also paralleled by changes in mean percent water since the pond L/pond H water ratio (Y) regressed against the sodium ratio (X) for the 9 weeks of observations is significant ($Y = 73.3\ X^{.068}$, $r^2 = 0.752$, $P < 0.05$).

Field sodium loss - Since tadpoles were not individually marked, the CPM's contained in the tadpoles of each treatment were averaged before and after exposure. Table 9 contains the initial and final mean CPM, and the percent reduction in mean CPM/day for each treatment. Two conclusions may be gathered from these data. (1) Pond L tadpoles were only 1.5 times larger (and contained 1.4 times more total sodium) than tadpoles from pond H, but took up 3.5 times more ^{24}Na. (2) Tadpoles from both pond H and pond L lost a higher (but different) percentage of their initial CPM's in pond L than in pond H.

Laboratory pH tolerance tests - During 4 weeks, *R. sylvatica* tadpoles from pond L survived significantly longer than those from pond H in pH 3.0 solutions; the reverse was true in 1 week and no difference was found in the other 4 weeks (Figure 13). There was no obvious trend in survival times of *R. sylvatica* tadpoles during development. The mean % reduction in body sodium concentration at the time of death was 45% and 37% for pond H and pond L tadpoles, respectively (not significantly different). Since tadpoles from pond L initially contained less sodium and lost similar amounts to tadpoles from pond H, this resulted in significantly lower body concentration at death for tadpoles from pond L during 5 of 7 weeks (other 2 weeks not significantly different). Both groups of animals lost a mean of 6% of their body potassium concentration during survival time testing. Similarly, pond H tadpoles lost 42% of their body chloride while tadpoles from pond L lost 44%, resulting in a significantly lower chloride concentration for the initially lower tadpoles from pond L. Water content did not change significantly during survival time experiments.

In contrast, *R. pipiens* tadpoles showed a linear increase in survival time (ST = min) and thus pH tolerance during the first 8 weeks (T = days) of development ($ST = 32.4 + 1.5\ T$, $r^2 = 0.841$, $P < 0.001$).

3.4 DISCUSSION

This study has shown conclusively that, in terms of sodium balance, amphibian larvae respond to low pH as has been reported for fish (Packer and Dunson, 1970, 1972). Recently published observations of McDonald *et al.* (1985) concerning the effects of low pH on *R. clamitans* tadpoles also support this contention. When amphibians are placed in a low pH solution, sodium influx is depressed while sodium efflux is markedly accelerated. The result is a large decline in body sodium content. Animals die when body sodium reaches approximately 50% of initial levels. Potassium content does not change during exposure. In fish, excess hydrogen ions disrupt sodium balance through their action on gill membranes (McWilliams, 1983). This study did not investigate the exact location of loss, but possible locations would include both the gills and the integument.

Table 9. Loss of radioactive sodium (24-Na) from pond H (high pH) and pond L (low pH) tadpoles (*R. sylvatica*) placed in their own as well as each others native pond. Values represent mean ± SD.

Source of tadpoles	Measurement pond	Sample	CPM/tadpole (n=10)	% reduction in mean tadpole CPM/tadpole/day
H	H	Initial	180,456 ± 47,396	16.1
		Final	151,668 ± 53,233	
H	L[a]	Initial	204,629 ± 74,938	38.4
		Final	126,809 ± 54,366	
L	L[a]	Initial	684,716 ± 192,907	64.2
		Final	254,034 ± 167,094	
L	H	Initial	673,358 ± 159,841	9.7
		Final	609,546 ± 214,520	

[a] one tadpole died during the course of the experiment (n=9 in final measurement).

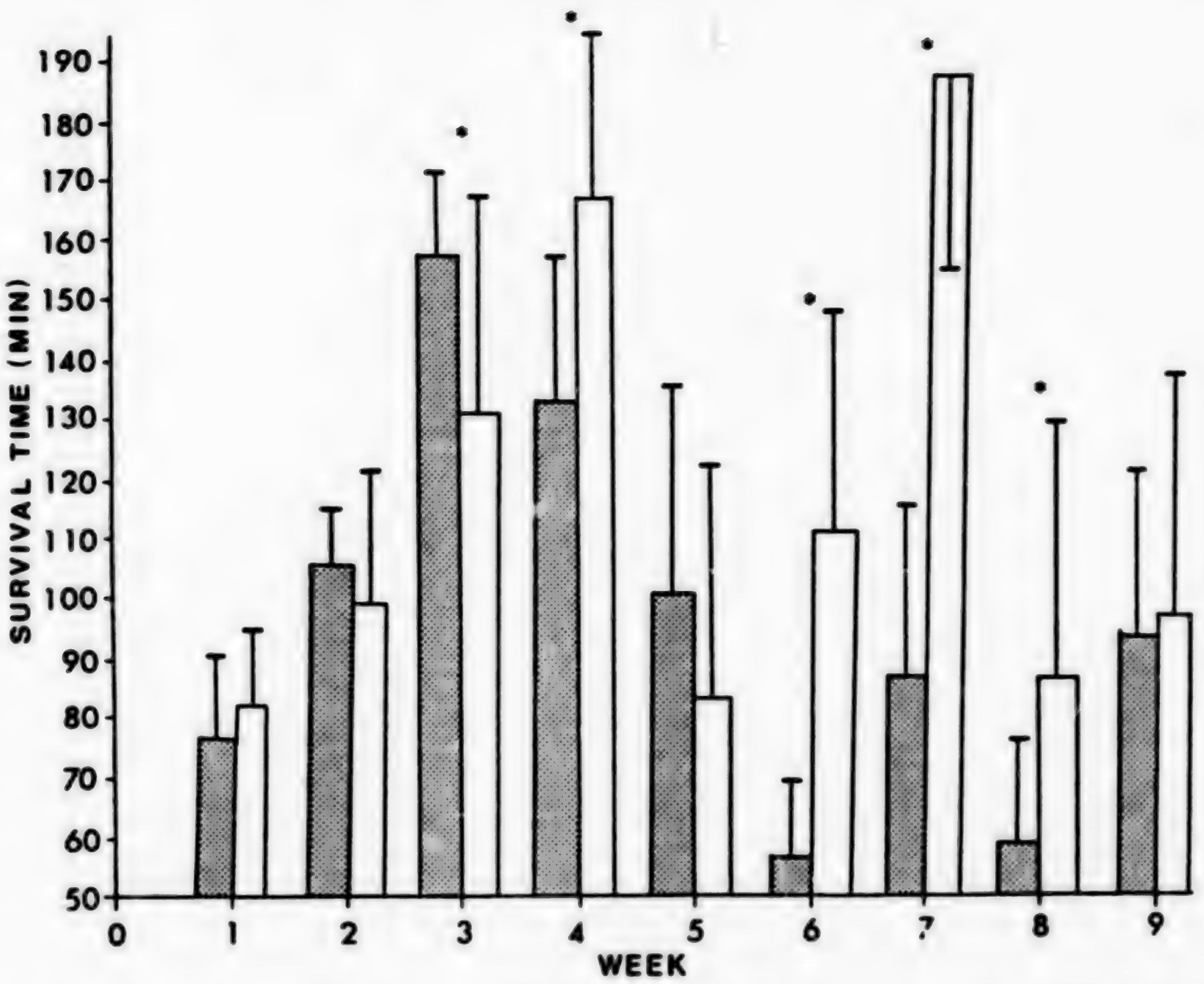

Figure 13. Mean survival time (±SD) of tadpoles (*R. sylvatica*) from ponds H (hatched bars) and pond L (clear bars) from hatching until metamorphosis in pH 3.0 artificial soft water solution (described in Table 1), (n = 10, asterisk indicates significant difference between ponds).

McWilliams (1982a) and Brown (1981) reported that increasing the calcium concentration in low pH solutions reduced sodium efflux of fish, and prolonged survival time. Sodium efflux from amphibians was also reduced upon increasing the external calcium concentration, resulting in increased survival times and a lower incipient lethal pH. Packer and Dunson (1970, 1972) reported that increasing sodium concentration in the bath to plasma levels increased survival time of brook trout in low pH solutions by 4 fold. We found that mean survival time of ten *R. pipiens* tadpoles in pH 3.5 was increased from 131 ± 129 min to 412 ± 6 min by increasing the NaCl concentration from 0.05 to 100 mM. As was reported for trout (Brown, 1981), neither potassium nor magnesium concentration (up to 5 mM) affected survival times of tadpoles in pH 3.0.

Amphibians chronically exposed to low pH also had a reduced body sodium content while potassium did not change. *R. pipiens* tadpoles in pH 4.0 solutions and *R. catesbeiana* in pH 3.5 solutions were extremely stressed and would have eventually died. They had lost 36 to 62% of initial body sodium content respectively. *R. catesbeiana* tadpoles can live for several weeks at pH 4.0, but still suffered a 21% reduction in sodium content after 7 days of exposure. The response of amphibians to low pH water is similar in some ways to sodium depletion resulting from maintenance in distilled water. Alvarado and Dietz (1970a) found that *Ambystoma gracile* could be maintained indefinitely in flowing distilled water but lost approximately 10% of its body sodium content; potassium content remained the same. When both larval and adult amphibians are maintained in distilled water, sodium loss rates are reduced, while sodium influx is greatly increased when animals are returned to water containing sodium (Alvarado and Dietz, 1970b; Greenwald, 1972). Amphibians whose sodium content was lowered by low environmental pH exhibited similar compensatory mechanisms. Influx rates rose significantly higher than controls in more basic pH's. Effluxes, although still higher than control levels, were significantly lower than loss rates during acute exposure. McDonald *et al*. (1985) did not observe acclimation in *R. clamitans* tadpoles pre-exposed to pH 5.0 water. This is understandable since *R. clamitans* can live at pH 3.5 and would not have been stressed by pH 5.0 water.

McWilliams (1982b) reported that the sensitivity difference to low pH exposure between two strains of brown trout could be attributed to the relative degree of disruption in sodium balance. McWilliams (1983) has also shown that calcium is leached from the gills of the sensitive strain at a faster rate, promoting higher rates of sodium loss. Interspecific sensitivity differences in amphibians are also equated with differences in the magnitude of sodium balance disruption. In our study, the acid tolerant *R. clamitans* had the highest influx rates at all pH's tested. Thus it could take up sizeable quantities of sodium at pH 4.0, while *R. catesbeiana* and *R. pipiens* could not. Slopes of regression lines of influx against pH were the same for all three species. The mode of inhibition of sodium influx by hydrogen ions is not known for amphibian gill. In fish, however, high external hydrogen ion concentration acts to inhibit the sodium-hydrogen exchange pathway (see review by Evans, 1975). The powerful inhibition of sodium influx observed in this study confirms the importance of this exchange mechanism in amphibian larvae.

Despite the inhibition of influx, we feel that mechanisms regulating sodium efflux are ultimately responsible for determining pH sensitivity. Accelerated sodium efflux is the probable cause of death in tadpoles during acute exposure to low pH and is quantitatively responsible for reductions in body sodium content during chronic acclimation. Individual differences in survival time of *A. opacum* at a specific pH reflected sodium loss rates.

Control animals (pH 6.0-7.0) in our study were not in sodium balance. Efflux was between 3.1 and 8.6 times higher than influx. Survival times of tadpoles in pH 3.0 solutions were also shorter during flux experiments than if left undisturbed. These discrepancies are probably due to handling stress. Influx and efflux of *R. pipiens* tadpoles after one continuous 2 h measurement period were not significantly different than after 4 30-min periods, but efflux did decline and become equal to influx if measurement periods were expanded to 24 to 48 h. Other studies (Alvarado and Dietz, 1970b; Alvarado and Moody, 1970) have also reported similar initially high rates of sodium loss which they attributed to handling stress.

It is interesting that normal body sodium content is markedly different among anuran species and is directly proportional to pH sensitivity. The difference between the species is so great that *R. clamitans* is operating in a range of body sodium concentrations that would be fatal to *R. pipiens*. In fish, body sodium content is also related directly to low pH sensitivity. Dunson *et al.* (1977) measured lower body sodium contents in the extremely tolerant cardinal tetra in comparison with guppies; brook trout are intermediate in both sensitivity and body sodium content (Packer and Dunson, 1970). It is tempting to speculate that amphibian tadpoles and larvae regulate their different body sodium contents as a specific adaptation for low pH. However, there are substantial intraspecific changes in both body sodium content and sensitivity during development that need further study.

The results of this study demonstrate that amphibians in the field are affected physiologically in a naturally low pH environment. Body sodium concentrations in *R. sylvatica* tadpoles from pond L were on the average 29.4% lower than tadpoles from pond H, with no apparent trend toward lower potassium concentrations. This situation would be predicted by the results of our laboratory experiments. The significantly lower body chloride concentration in pond L tadpoles also agrees with studies showing a disruption of chloride balance in fish exposed to low pH (McDonald *et al.*, 1980; McWilliams, 1980).

The higher unidirectional loss rate of sodium ions from tadpoles from either pond when placed in the low pH pond L, also indicates a detrimental interaction with sodium balance. Interpretation of the field sodium loss experiment is made simpler by comparison with related laboratory data. We have shown that *R. pipiens* tadpoles placed at pH 4.5 for 1 week have a higher sodium influx at pH 4.5 than those held at pH 5.8 prior to testing at pH 5.8. After low pH exposure, sodium efflux in *R. pipiens* at low pH declined slightly. This acclimation effect explains why tadpoles from pond L took up greater quantities of ^{24}Na in the loading bath and lost a smaller percent of their initial CPM's in the higher pH pond H, than did tadpoles from pond H. Tadpoles from ponds H and L had considerably higher sodium loss rates in pond L (38 and 64% respectively) in comparison with pond H (16 and 10% respectively). While it is impossible to say that tadpoles placed in pond L experienced a net sodium loss, it is reasonable to assume that the elevated efflux is a severe challenge to sodium balance. The large difference in percent CPM reduction between tadpoles from ponds L and H placed in pond L is difficult to interpret without more precise measurements on individual animals.

The decreased water concentration of tadpoles from pond L is in agreement with findings of McDonald *et al*. (1980) that trout exposed to low pH exhibit decreased plasma volumes. In an effort to maintain a constant extracellular osmotic pressure, tadpoles most likely compensated for lower body sodium concentration by excreting water.

The low pH of pond L was probably the main factor responsible for lower body sodium concentrations and higher sodium efflux. Many other variables differed slightly, but it is unlikely that they exerted any influence on sodium balance. Increased calcium concentrations reduce sodium efflux in both amphibians and fish (McWilliams, 1982a), but it is unlikely that the extremely small difference of only 2.7 mg/L during week 4 (3.2 mg/L mean difference for the 9 weeks) was the cause of the much higher sodium effluxes observed in pond L. Survival time of *R. sylvatica* tadpoles in pH 3.0 solutions does not significantly increase until the calcium concentration reaches 40 mg/L (Freda and Dunson, unpub. obs.). Survival time of a tadpole in acidic water is governed by its rate of net sodium loss (Chapter 3) and it may be assumed that this did not change appreciably until the calcium concentration was increased to 40 mg/L. It is also unlikely that the lower calcium concentration in pond L reduced body water content since Oduleye (1975) found that an increased calcium concentration (5 mM) in the external medium reduced body water content. Chronic exposure of trout to different calcium levels does not change plasma ion concentrations (McDonald *et al*., 1980). Quantitative differences in the concentration of organic compounds between the two ponds was probably also not a factor. The present study has shown that organic compounds in the water do not affect sodium influx or efflux. Other variables such as drying rate of ponds, and feeding were probably unimportant in relation to the lower body sodium concentration of tadpoles from pond L. Body sodium concentration was already lower in pond L within one week from hatching, when the tadpoles had not begun feeding and both ponds were full.

Although tadpoles from pond L lived significantly longer in lethally acidic solutions for 4 of the 8 weeks, we feel the data are inconclusive regarding the relative pH tolerance of the two populations of *R. sylvatica* tadpoles. No consistent trend in survival times of both groups of tadpoles was observed as they increased in age. The test pH (3.0) was lower than any possible pond pH's, yet it was possible to detect a linear increase in survival time during development in *R. pipiens* at pH 3.0, a species considerably less tolerant than *R. sylvatica*. A possible explanation for the lack of change in survival time with age in *R. sylvatica* may be differences in field acclimation temperature in relation to test temperature. Amphibian embryos are more sensitive to low pH at higher temperatures (Pough and Wilson, 1977) as are trout (Robinson *et al*., 1976). During the course of this study, pond temperatures gradually increased while the tolerance test was carried out in the laboratory at 22 C. The *R. pipiens* tadpoles were raised and tested in the laboratory at a constant 22 C.

4.0 POND CHEMISTRY

4.1 INTRODUCTION

Although much information has accumulated concerning the sensitivity of amphibian embryos of many species to acidic water, our knowledge of actual chemical conditions in breeding ponds remains incomplete. Toxicity of acidified water is a complex issue and detailed information is needed concerning pH, temperature, and the concentrations of aluminum, other ions (Na, Ca, SO_4, etc.) and organic compounds. Only one study has thus far collected such information (Dale et al., 1985). Our water chemistry studies were designed to provide descriptions in central and northeastern Pennsylvania. An exact determination of the sources of acidity in low pH ponds was beyond the scope of this study and would require further investigation.

4.2 METHODS

Study Ponds - We located amphibian breeding ponds in Pennsylvania by examining USGS topographic maps. Temporary forest ponds were studied in the ridge and valley province of the Appalachian Mountains (Rothrock State Forest and Pennsylvania Gamelands #182, Centre County), and on the Pocono Plateau (Delaware Water Gap National Recreation Area, Pike and Monroe Counties).

Sampling Protocol - Thirty-nine ponds in Centre County were sampled only once. From among these 16 ponds were selected for weekly sampling over periods varying in duration between March, 1983, and May, 1984. Selection of ponds for detailed study was made on the basis of their pH. Our objective was to obtain detailed information on a group of ponds representing the range of pond pH's found in central Pennsylvania. Thirty-five ponds in the Delaware Water Gap Recreation area were sampled once for field pH's during the spring of 1985. Pond names refer to the topographic map (7.5 quadrangle) on which it is located (except Alan Seeger and Bear Meadows which are on the Barrville and McAlevy maps, respectively).

Chemical Measurements - We measured pH in the field with a Leeds and Northrup model 7417 portable pH meter, and in the laboratory (within 12 hours) with a Beckman model 4500 pH meter. Field and laboratory pH measurements were usually less than 0.1 units apart, except during cold weather (< 5C) which adversely affected the field instrument. Laboratory values are reported hereafter except for Delaware Water Gap Ponds. During the spring of 1983, a pilot study determined the spatial variability of pH within 4 ponds ranging in pH from 4.1 to 5.5. pH was measured along the periphery of the pond and at its center; both surface and bottom water samples were collected. These measurements were taken twice; once during clear weather and again during a rainstorm. Water pH was found not to vary by more than 0.1 unit, regardless of weather conditions. Water was collected from the center of each pond, 5 cm below the surface. Sampling locations were usually within 1 meter of amphibian egg masses. Water samples were collected in acid-rinsed 500 ml polyethylene bottles, and stored at 4 C until analysis (within 36 h). Surface water temperature was measured with a mercury thermometer at the collection site. All samples were filtered through a 0.45 m Millipore filter after determination of pH. Alkalinity of unfiltered subsamples was determined by double endpoint titration (APHA et al., 1975). Concentrations of Na, K, Mg, and Ca were measured by atomic absorption spectrophotometry (Varian Techtron Model 1280). Total dissolved aluminum was measured with a graphite furnace. Samples were first filtered and then acidified with concentrated nitric acid (Al determinations only). Chloride concentration was measured by titration with mercuric nitrate, and sulfate with the barium chloride turbidimetric method (APHA et al., 1975). Tannin-lignin concentration was determined with a HACH tannin-lignin test kit, and expressed as mg/L tannic acid (HACH, 1973). Nitrate was determined by the cadmium reduction method (APHA et al., 1975). All data meet quality assurance criteria suggested by EPA (1979).

4.3 RESULTS

The results of our pond surveys in central Pennsylvania and the Delaware Water Gap (Northeastern PA) are presented in Appendix A and B, respectively. Geographic coordinates of these ponds are reported in Appendix C. Our laboratory and field bioassays indicate that pH's below 4.5 are deleterious to *Ambystoma* salamander embryos while wood frog embryos (*Rana sylvatica*) can generally survive in water with pH's as low as 4.1. Using these criteria, 6 of 39 (15%) central Pennsylvania ponds are detrimental to the development of *Ambystoma* salamander embryos; 4 of 35 (11%) ponds in Delaware Water Gap also fall into this category. Only one Delaware Water Gap pond was acidic enough to theoretically cause mortality of wood frog embryos.

It is difficult to make generalizations concerning temporal variation in pond chemistry because individual ponds were very different physically, chemically, and biologically. Ponds which experienced large volume changes due to drying (evaporation) and refilling (rain) underwent large (and variable) fluctuations in pH and ion concentrations. Permanent ponds were the most chemically stable. Despite this inter-pond variability a few generalizations are possible. Pond pH's were lowest immediately after melting and they gradually increased by 0.1 to 1.0 pH units during spring and summer. Correspondingly, alkalinity also increased as spring and summer progressed. Ponds also became darker (higher in tannic acid content) over time, probably due to the leaching of organic compounds from their leaf covered bottoms. Water hardness was positively correlated with pond pH and Al concentration increased dramatically with declining pond pH.

Ponds can be classified into 3 broad categories: 1) very low pH (3.80-4.50), clear water (less than 1 mg/L tannic acid) sphagnum ponds; 2) moderately low pH (4.50-5.50), dark water (1-10 mg/L tannic acid) ponds; and 3) high pH (5.5-8.55) ponds. Category 1 ponds were always lined with or surrounded by sphagnum moss. These ponds were typically the most dilute and had very clear water. When tannic acid concentrations exceeded 1 mg/L, the water became visibly stained. Category 2 ponds were very dark and visibility was reduced to a few cm in most cases. The high pH of category 3 ponds was usually attributable to anthropogenic influence. These ponds usually received runoff from nearby gravel roads or agricultural areas.

Precipitation did not have a directional effect on pond pH or alkalinity. Values for rain pH, amount and H+ deposition were obtained from the National Atmospheric Deposition Program (NADP) for the Leading Ridge site (Table 10). During 1983 and 1984, neither the pH or alkalinity of ponds sampled weekly (see Appendix A) were significantly correlated with the H+ deposition

Table 10. NADP rain data for the Leading Ridge, PA, site.

Period	Amount (in.)	Field pH	H^+ Deposition (mg/m^2)
3/08-3/15	0.52	4.14	0.67
3/16-3/22	2.43	4.56	1.35
3/23-3/29	1.02	4.44	0.69
3/30-4/05	0.71	4.24	0.85
4/06-4/12	2.05	4.02	3.43
4/13-4/19	0.79	4.28	0.85
4/20-4/26	1.75	4.67	0.72
4/27-5/03	2.76	4.26	3.47
5/04-5/10	0.42	4.14	0.57
5/11-5/17	1.05	4.03	1.62
5/18-5/24	2.36	4.25	2.58
5/25-5/31	0.67	4.05	1.33
6/01-6/07	1.00	3.90	2.68
6/08-6/14	0	-	0
6/15-6/21	2.13	3.82	5.45
6/22-6/28	> 0.01	3.54	1.31
6/29-7/05	0.57	3.97	1.06
7/06-7/12	0	-	0
7/13-7/19	0	-	0
7/20-7/26	1.32	3.71	4.76
7/27-8/02	0.43	3.67	1.44
3/20-3/27	0.70	4.28	0.65
3/28-4/03	1.74	4.46	1.23
4/04-4/10	0.93	4.07	1.80
4/11-4/17	0.78	4.08	1.25
4/17-4/24	0.40	3.94	0.88
4/24-5/01	0.33	4.23	0.46

(mg/m^2) which occurred during the previous 7 day period. Since pond pH's generally rose as spring and summer progressed, we regressed weekly changes in pH and alkalinity against H+ deposition to see if acidic precipitation had slowed the characteristic temporal rise in pH. Again, no significant correlations were found. Because our rain and pond chemistry data were collected weekly, we may have missed episodic declines in pH of short duration. During 1984 we analyzed pond water on a daily basis immediately before, during, and after a large rainstorm (April 4). This was the only large storm to occur while embryos were present during the spring of 1984. Of the 7 ponds sampled, only 4 could be sampled on April 3 before the rain began. All ponds were sampled on April 4, during the early portion of the rainstorm (0.11 in.), and on April 5, after the completion of the precipitation event (total = 0.86 in.). pH increased slightly (0.1 unit) or did not change in 3 of the 7 ponds (Table 11). The pH of the remaining 4 ponds declined by 0.1 to 0.2 units, but this decline is no larger than normal variation which occurred during dry periods. Alkalinity did not become depressed, but aluminum increased slightly (0.01 to 0.10 mg/L) in 5 ponds.

4.4 DISCUSSION

Our study provides valuable information regarding the range of chemical conditions in ponds of central and northeastern Pennsylvania, but is inconclusive as to the source of acidity in low pH ponds. The determination of the origins of acidity in soil and water (natural vs. anthropogenic) is an extremely complex topic and is currently the subject of much debate (Krug and Frink, 1983; LaZerte and Dillon, 1984). Pough and Cook observed large depressions in pond pH (0.25 to 0.75 units) after rainstorms. Temporal variation in the pH of our ponds was not correlated with weekly hydrogen ion deposition. Four of 7 ponds did however decrease slightly in pH (0.1 to 0.2 units) after a rainstorm, but this depression was within the limits of background variation. Even if our ponds exhibited large depressions in pH (> .5 units) after rains, this occurrence would not rigorously confirm acid precipitation as the cause. Percolation of water through peat deposits or water-saturated organic soils can significantly lower pond pH (Cronan et al., 1978; Hemond, 1980; Dawson et al., 1981). Many other natural factors may have been responsible for the acidic nature of particular ponds. For example, it is striking that all ponds with a pH below 4.5 were associated with large growths of sphagnum moss. Sphagnum can acidify small bodies of water through the exchange of endogenous hydrogen ions or environmental calcium or magnesium (Clymo, 1967). Two alternative perspectives are that sphagnum has invaded anthropogenically acidified ponds or the sphagnum alters the chemical conditions of the pond making it more susceptible to acid rain. The high concentrations of organic compounds in ponds also cannot be ignored and may be contributing to the acidity of many ponds (Hemond, 1980; Kilhan, 1982). The observation that many ponds maintained a pH below that of the rain also indicates a possible contribution from "natural factors". A strong piece of evidence implicating acidic precipitation is that pond pH's were lowest in spring after snowmelt, but again this is not conclusive. Pond pH's would be expected to rise naturally during late spring due to higher temperatures and biological activity. In addition, the average snowmelt pH is 4.5 (James Lynch, per. com.); well above the initial (spring) pH of many ponds.

The majority of ponds in central Pennsylvania were very dilute and exhibited extremely low alkalinities. These characteristics are associated

Table 11. Summary of water pH, alkalinity, and aluminum concentration in 7 central Pennsylvania amphibian breeding sites samples in 1984 before (4/3), during (4/4), and after (4/5) a rainstorm.

Pond	pH			Alkalinity ($mg/L\ CaCO_3$)			Total Dissolved Al (mg/L)		
	4/03	4/04	4/05	4/03	4/04	4/05	4/03	4/04	4/05
Barrville 2	-	4.60	4.50	-	-0.5	-1.6	-	0.09	0.14
Barrville 3	-	4.50	4.50	-	-0.5	-1.6	-	0.12	0.15
Bear Meadows	4.29	4.10	4.20	0	0	0	0.09	0.14	0.10
Julian 5	5.20	5.30	5.20	1.5	1.0	1.3	0.07	0.08	0.07
Julian 6	4.59	4.70	4.50	-0.5	-2.5	-1.5	0.17	0.16	0.15
McAlevy 1	-	4.20	4.20	-	0	0	-	0.60	0.60
McAlevy 7	4.50	4.30	4.30	-0.5	-0.5	-0.6	0.30	0.40	0.40

with a high sensitivity to acidic precipitation. Since a significant proportion of ponds we examined were low enough in pH to cause direct mortality of embryos, further studies should be initiated to determine the precise contribution of acidic precipitation. The small size of temporary ponds makes them ideal for the detailed study of hydrologic budgets and ion fluxes.

5.0 ECOLOGICAL EFFECTS OF LOW pH ON AMPHIBIANS

5.1 INTRODUCTION

The effect of low environmental pH on aquatic species distribution and abundance has been the focus of attention recently because of concern over effects of acidic precipitation (Haines, 1981). However, acidic waters occur naturally and undoubtedly have led to the evolution of adaptations to low pH. The bog lakes of Northern Minnesota, Wisconsin and Michigan (Jewell and Brown, 1929; Karns, 1984; Rahel, 1984), and the ponds and streams of the Atlantic coastal plain (Patrick *et al.*, 1979) are naturally acidic and toxic to species of amphibians sensitive to low pH (Gosner and Black, 1957). Other acidic locations include sphagnum bogs and temporary ponds in the Appalachians (Saber and Dunson, 1978; Chapter 4). Gosner and Black (1957) were the first to demonstrate interspecific differences in embryonic tolerance to low pH. Species sensitive to low pH were only observed in localized areas of high pH water within the generally acidic New Jersey Pine Barrens. In central Pennsylvania, many locally abundant species of amphibians were absent from an acidic sphagnum bog (Saber and Dunson, 1978). Beebee and Griffin (1977) found that the natterjack toad did not breed in acidic heathland ponds in England. Wilbur (1980) has also commented that low pond pH may be influencing local distributions of amphibians in the sandhills region of North Carolina.

The purpose of this study was to define the chemical environment experienced by embryos and larvae of amphibians and describe how it affects their survival and growth. We performed transplantations to determine if species intolerant of low pH are prevented from successfully breeding in acidic ponds. The possible inhibition of growth of tadpoles by low pH was also investigated.

5.2 METHODS

Study ponds - We located amphibian breeding ponds by examining USGS topographic maps. We conducted surveys to determine the existing range of pond pH's. A subset of 8 to 12 ponds was selected to test hypotheses concerning hatching success. Study ponds were selected on the basis of pH to provide an acidity gradient representative of the entire range of pond pH's in central Pennsylvania. We studied temporary forest ponds in the ridge and valley province of the Appalachian Mountains (Rothrock State Forest and Pennsylvania Gamelands #182, Centre County) and Bear Meadows, an acidic sphagnum bog studied by Saber and Dunson (1978). The temporary ponds generally dried in late summer or autumn and refilled during winter or early spring from rain or melting snow. The Jefferson salamander (*Ambystoma jeffersonianum*) and the wood frog (*Rana sylvatica*) were studied.

Chemical Measurements - We analyzed pond water weekly during the period of breeding and embryonic development. A detailed description of analytical methods is presented in the previous chapter.

Amphibian Survey - We searched ponds weekly for egg masses from 20 March to 1 May 1984. Because egg masses of *R. sylvatica* and *A. jeffersonianum* tended to be either very abundant or absent, we classified each species as present or not. Percent mortality of naturally laid egg masses was not measured because embryos were usually healthy if present. We did however examine 6 to 12 egg masses just prior to hatching to determine if any embryos suffered from the hatching defect.

Laboratory Bioassay Tests - We performed a series of toxicity tests to determine the lowest pH at which embryos of each species could hatch. We collected 12 egg masses of *A. jeffersonianum* from pond Julian 6 (26 March, 1984) and *R. sylvatica* from pond McAlevy (4 April, 1984). All embryos were at either 1 or 2 cell stage. These locations are the lowest in pH known to support breeding for each species in central Pennsylvania. If genetic differences in tolerance to low pH exist between animals taken from different ponds within a contiguous area, our choice of acidic donor ponds should lead to the most conservative estimates of mortality. Egg masses were divided into pieces containing 5 to 7 embryos and placed into bowls containing their native pond water. Groups of twenty embryos were then randomly chosen and placed in 1 L of artificial water (ASW, Table 12) adjusted to one of several pH's with dilute sulfuric acid. Embryos were kept at 9 C under a 12:12 photoperiod. The pH was checked daily and adjusted if it changed by more than 0.05 units; drift rarely exceeded 0.05 units per day. Dead embryos were not removed, mimicing the natural situation. We recorded larvae as hatched if they successfully escaped from their embryonic membranes and exhibited no gross morphological abnormalities.

Additional toxicity tests were performed to determine if darkly stained bog water was more toxic than clear water solutions, of similar pH's and ion concentrations. Pine Barrens tree frog (*Hyla andersoni*) and Fowler's toad (*Bufo woodhousei*) embryos were collected in Ocean County, N.J., and embryos of the green frog (*R. clamitans*) from Bear Meadows Bog. *Hyla andersoni* and *B. woodhousei* were incubated in naturally acidic, darkly stained Carpenter Pond water (CPW, Table 13), and *R. clamitans* was incubated in Bear Meadows Water (Table 13). Parallel incubations were also conducted in ASW at a similar pH (22 C).

Field Transplantation Experiments - Transplantation experiments during the spring and summer of 1984 tested whether species missing from acidic ponds were excluded by embryonic mortality. In Pennsylvania, egg masses were collected from the same two donor ponds used for laboratory bioassays (on the same dates), and were separated into pieces containing approximately 15 to 20 embryos each as previously described. Approximately 100 embryos were then placed in 14 X 18 X 7 cm nylon screen cages. Four replicates for each species were placed in each of 12 temporary ponds where some egg deposition naturally occurs. We examined embryos weekly for abnormalities characteristic of low pH exposure (yolk plug extrusion, curling defect, etc.). All larvae were counted after hatching.

Growth Experiments - We investigated sublethal effects of low pH on tadpoles of *B. woodhousei* and *H. andersoni* by raising them in baths of different pH's. Five newly hatched tadpoles were placed in 5 L of Webbs Mills water (Table 13) adjusted to specific pH's (3 replicates) and given 0.1 g of commercial trout chow. Food and water were replenished weekly. The food ration was increased to 0.2 g at the start of week 3, so that it was always in excess. Tadpoles of *Hyla andersoni* were raised at pH's 3.75, 4.00, 4.50 and 6.00. Tadpoles of *B. woodhousei* were raised at pH's 4.10, 4.50, and 6.00. The pH was adjusted daily with dilute H_2SO_4 or NH_4OH (pH 6.0 bowls

Table 12. Chemical composition of water used for embryo bioassays and tadpole growth experiments.

Water	pH	Na	K	Mg	Ca	Al	Cl	Tannic Acid
Artificial Soft Water (ASW)	5.80	1.0	1.0	0	1.0	0	4.2	0
Bear Meadows	4.28	0.8	0.8	0.6	0.9		3.3	6.1
Carpenter Pond (CPW)	4.10	1.1	1.1	0.3	1.0		3.2	5.1
Webbs Mills								
mean	4.43	1.8	0.2	0.4	0.4	0.07	3.8	1.3
range	4.3-4.5	1.5-2.1	0.2-0.3	0.2-0.6	0.3-0.8	0.06-0.08	2.6-4.2	1.1-1.4

Table 13. Mean percent hatching of *Ambystoma jeffersonianum* and *Rana sylvatica* embryos (n=20) in ASW adjusted to different pH's. Asterisk (*) indicates significant difference between *A. jeffersonianum* and *R. sylvatica* (chi square test: = 0.05).

pH	Ambystoma jeffersonianum		Rana sylvatica
5.80	100%		95%
4.75	100%		--
4.50	25%	*	100%
4.25	0%	*	80%
4.00	0%		0%

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only). Drift of pH was minimal during the first 2 weeks (< 0.1 units) but increased to 0.1–0.3 units per day during week 3. Tadpoles were kept at room temperature (22-26 C) and ambient illumination, and were weighed in a beaker of water on a Mettler Balance. The effect of low pH on growth of *R. pipiens* tadpoles was also studied. Five 3-day old (stage 25) tadpoles were placed in 5 L of 5% tap water. Six replicates were used; half were adjusted to pH 4.4 while the remainder were left unadjusted (started at pH 5.8). The pH of the 4.4 bowls was adjusted twice daily and generally drifted upwards approximately 0.3 to 0.4 units. The water was changed and 1 g of Nasco frog brittle was added 3 times a week. The pH of the unadjusted bowls drifted upwards by approximately 0.5 units between changes.

Statistical Analyses - The effect of pH on hatching of each species, the difference in hatching between species in ASW, and intraspecific differences between ASW and natural bog water at a single pH were analyzed with the chi square statistic ($\alpha = 0.05$). The presence or absence of each species in breeding ponds was analyzed by comparing the median pH's of ponds with and without each species (Mann Whitney U Test, $\alpha = 0.05$). Page's one way test for ordered alternatives ($\alpha = 0.05$) was used to determine if hatching of embryos was reduced as pond pH's became progressively lower. A significant difference between the hatching of the 2 species in each pond was determined with a Mann Whitney U Test ($\alpha = 0.05$). The relationship between decreasing pH and the size and growth rate of tadpoles was analyzed with a Page's one way test for ordered alternatives ($\alpha = 0.05$).

5.3 RESULTS

Laboratory Bioassays - Mortality of embryos significantly increased as pH declined (Table 13). *Ambystoma jeffersonianum* was significantly more sensitive to low pH than *R. sylvatica*. Only 25% of the *A. jeffersonianum* embryos hatched at pH 4.50, while 100% and 80% of the embryos of *R. sylvatica* hatched at pH 4.50 and 4.25, respectively. Approximately one half of the embryos of *A. jeffersonianum* killed at pH 4.50 were tightly curled; the remaining half stopped developing after transfer to low pH. All embryos of this species placed at pH's 4.25 and 4.00 died immediately. Twenty percent of the embryos of *R. sylvatica* at pH 4.25 had nodule-like growths covering their external surface and failed to hatch; at pH 4.00, all embryos stopped developing soon after transfer.

Hatching of *H. andersoni* and *B. woodhousei* was significantly reduced over a very small range in pH (< 0.2 units, Table 14). Significantly more *H. andersoni* hatched at pH's 3.75 to 4.00 than *B. woodhousei*. *H. andersoni* evidenced 90% hatching in pH 3.80, while *B. woodhousei* did not hatch below pH 4.10. All 3 species tested had significantly lower hatching in natural "dark" water compared to ASW (Table 14). Embryos of *H. andersoni* in pH 4.10 CPW became curled, but eventually hatched; those in pH 3.75 CPW died from the curling defect. Embryos of *H. andersoni* in ASW never became curled; those that died at pH 3.70 and 3.75 stopped developing after transfer. *Bufo woodhousei* embryos killed in CPW also died from the curling defect, while those in ASW die early in development. Embryos of *R. clamitans* incubated in bog water exhibited a greater degree of curling and a significantly higher mortality than those held in ASW.

Table 14. Percent hatching of *Bufo woodhousei*, *Hyla andersoni* and *Rana clamitans* in ASW and natural bog water. Sample size is given in parentheses and asterisks (*) indicate significant difference between ASW and CPW at a specific pH. Double asterisks (**) indicate significant difference between *B. woodhousei* and *H. andersoni* in ASW at a specific pH (chi square test: $\alpha = 0.05$).

	Bufo woodhousei		*Hyla andersoni*		*Rana clamitans*	
pH	ASW	Carpenter Pond	ASW	Carpenter Pond	ASW	Bear Meadows
5.8	100% (40)		100% (40)			
4.50	98% (40)		100% (60)		100% (20)	
4.30	100% (40)					
4.25	100% (40)		90% (40)		100% (20)*	35% (20)
4.20	98% (40)					
4.10	85% (40)*	45% (40)		93% (40)		
4.00	0% (80)	0% (40)**	97% (60)*	85% (40)	100% (20)*	10% (20)
3.90	0% (40)	**	05% (20)			
3.80	0% (40)	**	90% (20)			
3.75	0% (40)	0% (40)**	25% (40)	20% (40)	0% (20)	
3.70			25% (20)			

ASW - Artificial Pond Water

CPW - Carpenter Pond Water

Field Survey - The median pH of ponds that contained embryos of *A. jeffersonianum* was significantly higher than those ponds without them. In support of laboratory toxicity tests, *A. jeffersonianum* was not observed in any ponds with a mean pH below 4.62, while the presence of the less sensitive *R. sylvatica* was unrelated to pond pH (Table 15). When present each species was always abundant with one exception. In pond Julian 20, one egg mass of *A. jeffersonianum* was found, but all embryos were curled.

Field Transplants - Percent hatching of embryos of *A. jeffersonianum* significantly declined as pond pH's decreased (Table 16). A detailed summary of pond chemistry during the incubation period is given in Table 17. No relationship existed between hatching of embryos of *R. sylvatica* and pond pH. The percentage of hatching of the 2 species was significantly different in 7 of the 12 ponds; *R. sylvatica* was 39 to 83% higher in 5 ponds, while *A. jeffersonianum* was only 9 and 12% higher in the other two. Hatching of embryos of *A. jeffersonianum* was low (0-38%) in 5 of the 6 ponds which did not naturally support breeding. Eighty-one % of transplanted embryos hatched successfully in pond McAlevy 7, but no naturally laid eggs were found there. Another inconsistency is that *A. jeffersonianum* bred successfully in Julian 8, yet most transplanted embryos died. In only 1 of 3 ponds that lacked *R. sylvatica*, was mortality elevated in transplanted embryos (Bear Meadows, 54% hatching). In ponds Julian 8 and Alan Seeger, naturally laid egg masses were healthy, but only 52 and 26% of the transplants hatched, respectively. *A. jeffersonianum* in Bear Meadows and McAlevy 1, and *R. sylvatica* in Bear Meadows were killed by the curling defect. Deaths in other ponds resulted from developmental abnormalities including thoracic swelling and yolk plug extrusion.

Effects of Low pH on Growth - pH had a significant effect on the size of tadpoles of *H. andersoni* ($P < 0.025$ for 0-12 and 12-26 days) and *B. woodhousei* ($P < 0.025$ for 0-10 and $P < 0.100$ for 10-26 days; Table 18). Growth was significantly reduced at lower pH's during the initial 10 days. Growth of *B. woodhousei* was reduced by 40% at pH 4.10 (lowest hatchable pH), while that of *H. andersoni* was reduced by 25% at pH 4.00 and 40% at pH 3.75 (lowest hatchable pH). Although no significant reduction of growth occurred during the following 2 weeks, size differences were still evident from earlier inhibition. Environmental pH had a significant effect on the growth rate of *R. pipiens* tadpoles (Fig. 14). Tadpoles at pH 4.4 and 5.8 grew at rates of 5.9 and 26.0 mg/day respectively, during the 24 day test period.

5.4 DISCUSSION

Implications for Amphibian Community Composition - This study has shown that the pH's of some Pennsylvania ponds are low enough to kill the low pH-sensitive embryos of *A. jeffersonianum*, while embryos of the more tolerant *R. sylvatica* were less affected. Many biotic and abiotic factors such as temperature, pond drying, competition, and predation can influence amphibian community composition (Wilbur, 1984). It would be naive to try to explain all variations in community composition by any single variable such as pH. However, mortality of embryos of a sensitive species due to low pH would obviously have a major influence on distribution and abundance in areas containing acidic ponds. We observed reasonable agreement between natural absence of a species from a particular pond, and the inability of transplants to hatch there or in ASW at a similar pH in the laboratory. The death of

Table 15. Presence (+) or absence (0) of *Ambystoma jeffersonianum* and *Rana sylvatica* embryos in 12 central Pennsylvania breeding sites during 1984.

Pond	Mean Pond pH	*Ambystoma jeffersonianum*	*Rana sylvatica*
Julian 1	7.06	+	0
Barrville 1	5.71	+	+
Julian 5	5.41	+	+
Julian 8	5.19	+	+
Barrville 2	4.92	+	+
Julian 6	4.75	+	+
Julian 20	4.62	+*	o
McAlevy 2	4.45	0	+
Alan Seeger	4.40	0	+
McAlevy 7	4.39	0	+
McAlevy 1	4.35	0	+
Bear Meadows	4.24	0	0

*one egg mass found but all embryos were curled

Table 16. Percent hatching of embryos of *Ambystoma jeffersonianum* and *Rana sylvatica* transplanted into 12 breeding sites in central Pennsylvania. Values represent the mean and SD of 4 replicates of approximately 100 embryos each. Asterisks (*) indicates a significant difference between values for *A. jeffersonianum* and *R. sylvatica* in the same pond (Mann Whitney U test: $\alpha = 0.05$).

		Ambystoma jeffersonianum		*Rana sylvatica*	
Pond	Mean Pond pH	Mean Hatching	SD (n)	Mean Hatching	SD (n)
Julian 1	7.06	81	8 (404)	77	6 (406)
Barrville 1	5.71	87	3 (411) *	78	7 (414)
Julian 5	5.41	79	9 (419)	80	15 (399)
Julian 8	5.19	7	8 (380) *	52	7 (409)
Barrville 2	4.92	91	3 (401) *	79	4 (447)
Julian 6	4.75	74	3 (358)	73	11 (378)
Julian 20	4.62	0	(402) *	83	3 (429)
McAlevy 2	4.45	38	10 (394) *	77	3 (406)
Alan Seeger	4.40	27	8 (392)	26	10 (409)
McAlevy 7	4.39	81	9 (427)	81	8 (400)
McAlevy 1	4.35	28	19 (380) *	77	7 (427)
Bear Meadows	4.24	12	8 (419) *	54	15 (409)

Table 17. Means and ranges of water chemistry variables of 12 amphibian breeding sites in central Pennsylvania. Samples were taken weekly from March 28 to April 25, 1984. Aluminum was measured only on April 4 and 11.

		Temp.				(mg/l)			Tannic
Pond	pH	(°C)	Na	K	Mg	Ca	Al	Cl	acid
Alan Seeger									
mean	4.40	9.3	0.6	0.3	0.4	1.7	0.22	1.2	0.4
range	4.25-4.60	8-11	0.2-0.9	0.2-0.4	0.3-0.5	1.5-1.9	0.18-0.28	1.0-1.4	0.3-0.4
Barrville 1									
mean	5.71	10.9	0.5	0.4	1.4	2.6	0.09	1.1	1.3
range	5.50-5.74	6-17	0.3-0.6	0.3-0.6	1.1-1.9	1.8-3.6	0.07-0.10	1.0-1.2	0.7-1.6
Barrville 2									
mean	4.92	9.8	0.4	0.9	0.9	2.2	0.10	1.2	1.8
range	4.60-5.27	3-17	0.2-0.6	0.6-1.1	0.8-0.9	1.4-2.8	0.09-0.11	0.9-1.6	0.5-2.6
Bear Meadows Bog									
mean	4.24	5.8	0.4	0.4	0.6	1.4	0.13	1.2	1.5
range	4.10-4.39	2-9	0.3-0.5	0.2-0.7	0.6-0.7	1.0-2.2	0.12-0.14	1.1-1.4	1.4-1.8
Julian 1									
mean	7.06	9.0	0.3	0.6	2.2	16.2	0.02	0.9	0.6
range	6.87-7.25	5-15	0.1-0.5	0.6-0.7	0.7-3.0	12.9-19.2	0.02-0.02	0.2-1.3	0.4-0.8
Julian 5									
mean	5.41	9.5	0.3	1.7	0.4	1.8	0.05	1.2	1.4
range	5.30-5.60	8-12	0.2-0.4	1.5-1.8	0.3-0.5	1.1-2.2	0.02-0.08	1.2-1.3	1.3-1.6
Julian 6									
mean	4.75	8.4	0.4	0.8	0.4	1.1	0.15	1.2	1.3
range	4.62-4.85	5.5-11	0.3-0.6	0.6-0.9	0.4-0.5	1.0-1.2	0.15-0.16	1.1-1.4	0.9-1.8
Julian 8									
mean	5.19	7.8	0.2	2.0	0.4	1.6	0.10	1.3	2.6
range	4.78-5.40	1-12	0.1-0.4	1.5-2.3	0.3-0.4	1.5-2.0	0.09-0.11	1.0-1.7	2.0-3.1
Julian 20									
mean	4.62	7.5	0.3	2.1	0.5	1.8	0.23	2.1	2.5
range	4.60-4.65	3-12	0.3-0.4	1.7-2.7	0.4-0.5	1.5-1.9	0.21-0.25	1.9-2.2	1.8-2.9
McAlevy 1									
mean	4.35	9.6	0.5	0.6	0.5	1.1	0.60	1.2	0.5
range	4.20-4.44	2-16	0.3-1.0	0.2-1.1	0.4-0.6	1.0-1.3	0.60-0.60	1.1-1.4	0.3-0.7
McAlevy 2									
mean	4.45	9.2	0.5	1.2	0.7	1.6	0.33	1.3	0.5
range	4.40-4.50	2-16	0.3-1.0	0.8-1.6	0.6-0.8	1.5-1.9	0.31-0.35	1.0-1.6	0.3-0.8
McAlevy 7									
mean	4.39	8.8	0.4	0.6	0.6	1.2	0.40	1.1	0.8
range	4.19-4.69	2-17	0.2-0.6	0.3-0.8	0.4-0.8	1.1-1.7	0.40-0.40	1.0-1.3	0.4-1.6

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Table 18. Size and growth of *Bufo woodhousei* and *Hyla andersoni* tadpoles maintained in Webbs Mills Water adjusted to different pH's (3 replicates of 5 tadpoles). Values represent mean ± SD.

Days	pH Treatment	Wet Mass, mg		Growth Rate, mg/day	
0	initial	8.7	0.3		
10	4.10	46.7	6.0	3.8	0.5
	4.50	56.5	10.5	4.8	0.3
	6.00	77.7	23.2	6.9	2.0
26	4.10	263.3	26.5	13.5	1.0
	4.50	279.2	33.3	13.9	0.6
	6.00	294.6	54.4	13.6	1.9
0	initial	15.3	3.9		
12	3.75	88.6	13.9	6.0	0.4
	4.00	112.6	24.4	8.1	1.2
	4.50	125.4	41.6	9.2	1.1
	6.00	148.7	51.6	11.1	3.8
26	3.75	182.2	55.5	6.9	2.6
	4.00	227.0	55.9	7.9	1.6
	4.50	229.8	98.3	7.5	1.8
	6.00	256.6	50.6	7.7	3.5

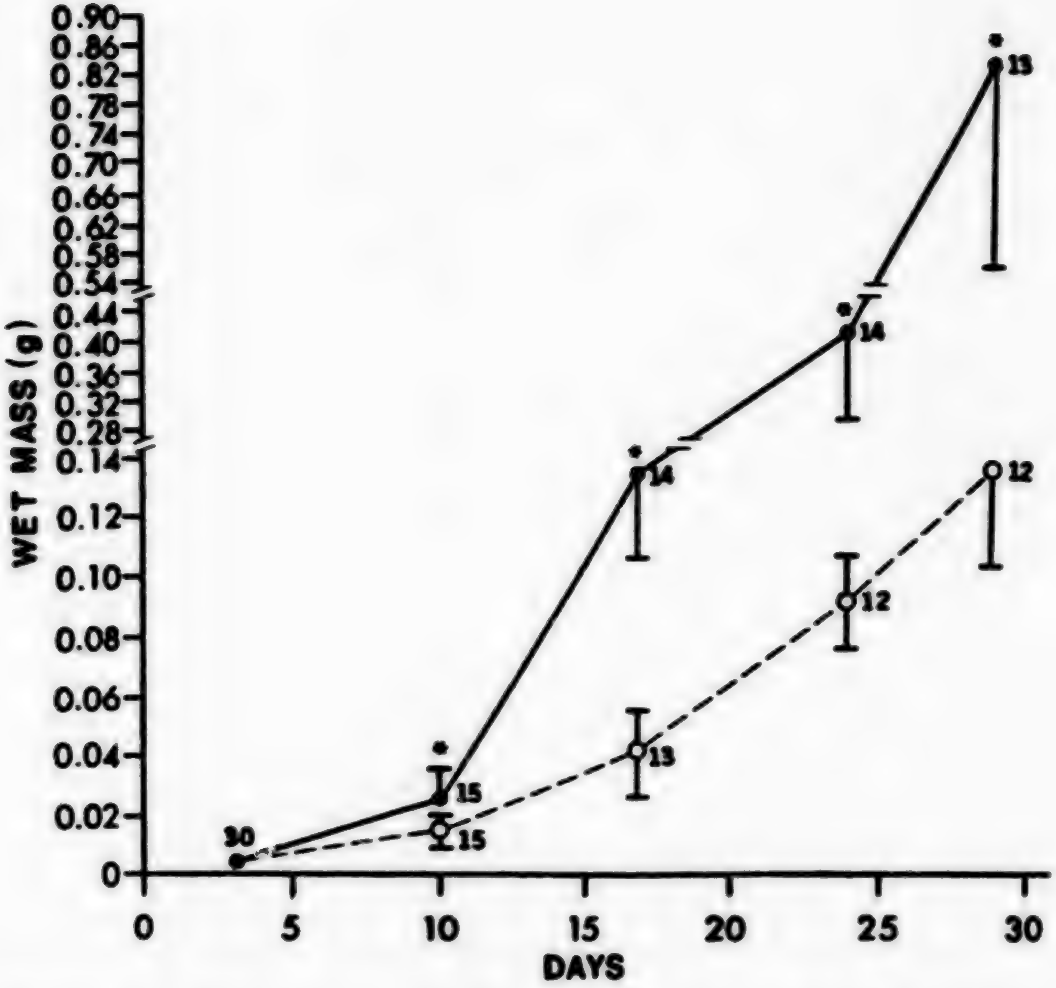

Figure 14. Mean wet mass (± SD) of *R. pipiens* tadpoles raised in either pH 4.4 (O) or 5.8 (O) [number adjacent to mass is sample size, asterisk indicates significant difference between treatments). Lines connect mean values.

embryos from deformities characteristic of low pH (yolk plug extrusion, swollen thorax, curling defect) tends to confirm low pH as the probable cause of death. Since transplanted embryos of R. sylvatica were capable of hatching in ponds Julian 1 and 20, the lack of natural breeding in these ponds is probably due to factors other than pH. Another similar case is the ability of embryos of A. jeffersonianum to hatch in pond McAlevy 7, where none occurred naturally. Numerous natural egg masses of the spotted salamander (A. maculatum) hatched in this same pond, but only 1 larvae was captured after 10 successive weekly samplings. Some abiotic or biotic factor(s) made this pond inhospitable to Ambystoma larvae. Pond Julian 8 contained live egg masses of A. jeffersonianum and R. sylvatica, and pond Alan Seeger had healthy eggs of R. sylvatica, but embryos transplanted into both ponds suffered high mortality. Possible explanations include different microhabitat placement of eggs, population differences in tolerance to existing environmental conditions, and experimental artifact.

This study intensively investigated 5 Pennsylvania ponds with pH's below 4.5, but we are aware of several others. At all these sites, A. jeffersonianum is missing and R. sylvatica is abundant. Rana sylvatica is generally considered very tolerant to low pH in comparison with Ambystoma. Tome and Pough (1982) reported no increase in mortality of embryos of R. sylvatica at pH 4.0, whereas A. jeffersonianum experienced 40% mortality. Pierce et al. (1984) and Gosner and Black (1957) also reported no mortality of R. sylvatica embryos at pH 4.0. We observed no hatching of R. sylvatica in ASW at pH 4.0. The apparently higher sensitivity of R. sylvatica and A. jeffersonianum in Pennsylvania may be due to genetic variation (Pierce and Sikland, 1985) and/or differences in the chemical composition of the test solutions or other experimental procedures (Chapter 2).

The failure of A. jeffersonianum to enter and oviposit in acidic ponds may result from two different mechanisms. Ambystoma maculatum salamanders have high fidelity to breeding ponds, thus high embryonic mortality may prevent colonization and subsequent breeding (Whitford and Vinegar, 1966). Adult salamanders may also be selecting against acidic ponds since they can differentiate between substrates of different pH (Mushinsky and Brodie, 1975).

Toxicity Due to Aspects Other Than pH - Although embryos of sensitive species generally suffered higher mortality in low pH ponds, mortality in ponds with similar pH's was highly variable. These inconsistencies probably indicate interaction between pH and other chemical variables. Bog water used in laboratory bioassays was significantly more toxic to amphibian embryos than ASW adjusted to identical pH's. Dunson and Connell (1982) reported the total inhibition of hatching of Xenopus laevis at pH 4.3 in Bear Meadows water and at pH 3.9 in "clear water" from Buffalo Run. Clark and Hall (1985) reported a strong negative correlation between dissolved organic carbon and hatching of 3 species of amphibians in stream pools of southern Ontario. Several of our observations also suggest an added toxic component to "dark water", in addition to low pH. In Pennsylvania ponds, the precise levels of mortality were slightly higher or lower than levels observed in laboratory trials in ASW. The low hatching success of A. jeffersonianum in ponds Julian 8 and 20, and that of R. sylvatica in pond Julian 8 may have been influenced by the high concentrations of organic compounds in these ponds.

Dissolved Al has also been shown to influence embryonic hatching success and its toxicity varies with pH and the inorganic and organic complexes that it forms (Clark and Hall, 1985). Aluminum can exist as free Al^{+++}, AlF, $Al(OH)_3$, or be organically bound (Driscoll, 1980; Perdue et al., 1976). The toxicity of Al to fish embryos, which are physiologically similar to amphibian embryos, is caused by Al^{+++} and $Al(OH)_3$. Aluminum fluoride is intermediate in toxicity and the organically bound form is harmless (Driscoll et al., 1980). Below pH 4.4, Al actually alleviates low pH stress. Yet above pH 4.4, Al becomes extremely toxic at concentrations of only 0.2 mg/L (Baker and Schofield, 1980). Therefore Al may not be toxic in "dark-water" ponds, while Al may either mitigate or increase low pH stress in "clear water" ponds, depending on the pH. Such complex interactions may contribute to the differences observed in mortality between field and laboratory bioassays. For example, 27 to 81% of transplanted A. jeffersonianum embryos hatched in the clear, acidic, high Al waters of ponds McAlevy 1, 2, 7 and Alan Seeger; no embryos hatched at comparable pH's in the laboratory. Thus hatching success of amphibians in acidic ponds is a result of complex interactions between temperature, pH, Al, organic compounds and perhaps certain other inorganic constituents. For species which take considerable time to hatch, these factors change over time, as does the intrinsic pH sensitivity of the embryos, which peaks at neurulation and hatching (Tome and Pough, 1982).

Inhibition of Growth - The slower growth of larvae in water low in pH has important ecological consequences. Wilbur (1984) states that growth of tadpoles is the most important determinant of larval survival. Slower growing individuals in temporary ponds may not metamorphose before drying of ponds. Smaller individuals are also more susceptible to gape-limited predators, such as adult Notophthalmus and Ambystoma larvae. Similarly, stunted gape-limited predators would presumably be less efficient since their range in prey sizes would be reduced. The outcome of intra- and interspecific competition is also heavily dependent on size. Physiological adaptations of species tolerant to low pH may have evolved in response to these interactions and may involve the use of acidic ponds as refuges from less tolerant competitors and predators. High mortality of embryos of sensitive species can be observed under natural conditions, but the indirect effects of low pH on competition and predation need to be rigorously tested in either caging or cattle tank experiments (Wilbur, 1984; Morin, 1983).

6.0 RESEARCH NEEDS

A significant percentage (11-15%) of temporary ponds in central and northeastern Pennsylvania are low enough in pH to cause direct embryonic or larval mortality of low pH sensitive species of amphibians (less than pH 4.5). An additional 16% of ponds were acidic enough (pH 4.5-5.0) to have sublethal effects on larvae, which may alter biotic interactions (competition and predation) and ultimately influence species distribution and abundance. However, we feel that there is no immediate threat of extinction or large scale reduction in numbers or range of amphibians which use central and northeastern Pennsylvania's temporary forest ponds. Extrapolation to other regions should be made with caution. Although weekly hydrogen ion loading was not correlated to pond pH and the one storm event studied did not have a large influence on pond pH, acidic precipitation may be having a negative impact on amphibian populations. Our methods could have missed small depressions in pond pH's (less than 0.2 units) due to temporal variation in pond chemistry and a poor understanding of the role of natural acidifying factors (sphagnum, organic acids). A very small decline in pond pH (0.2 units) can reduce hatching from 100% to 0% in species at the edge of their tolerance. Small sublethal depressions in pond pH may also be altering biotic interactions. We envision that an amphibian's local distribution and abundance are controlled by complex interactions between biotic relationships, environmental factors (such as pH, temperature, etc.), and the physiological and behavioral adaptations of each species. It is quite possible that acidic precipitation is having subtle influences on these processes, without causing large scale depressions in pond pH or direct mortality of amphibians. A great deal more information is needed before the impact of acidic precipitation on Pennsylvania's amphibians can be fully assessed. Specifically, studies investigating the effect of snowmelt and dry deposition on pond chemistry are needed. Detailed biogeochemical studies determining the relative importance of natural and anthropogenic contributions of acidity to pond chemistry would be essential.

Our physiological and toxicological studies will be very valuable in interpreting the impact of acidic deposition in other areas, but our regional assessment (PA) of acid rain effects probably cannot be extrapolated to a national level. Other investigators in New York and Massachusetts have observed large depressions in pond pH after rainstorms. Conflicting results of several toxicological and field studies also suggests considerable variation in tolerance of various species to low pH. Some standardization of laboratory bioassay techniques is badly needed. Any comparative studies require that different species and populations be tested in waters of identical chemistry. Detailed surveys and the establishment of chemical and biological data bases are badly needed in other areas of the United States. Two obvious areas would include the Adirondack mountains and southern Appalachians. The lakes and streams of these regions have been shown to be seriously impacted by acidic precipitation.

Our study and those of most others investigating the effects of acidic precipitation have focused narrowly on amphibians which breed in temporary ponds. Information is badly needed concerning the possible impact of acid rain on other groups of amphibians. For example, no information exists regarding the low pH tolerance of stream-dwelling Plethodontid salamanders, even though many studies have demonstrated large episodic depressions in pH and elevation in Al concentration in mountain streams after rainstorms or snowmelt (Corbett and Lynch, 1982; Dewalle et al., 1982).

In summary, our study has detected a significant number of ponds with chemical conditions (low pH and high Al concentration) detrimental to the survival of amphibians which breed in the temporary forest ponds in central and northeastern Pennsylvania. Acidic precipitation may be the major influence on pond pH, but further detailed studies are needed to determine its exact contribution to pond acidity. Most ponds studied had extremely low alkalinities and are therefore theoretically susceptible to acidification by rain. The certain continuation of acidic precipitation could eventually deplete current sources of soil buffering causing large scale declines in pond pH. Small declines (on the order of tenths of a pH unit) can totally block embryonic development of amphibians. Thus there is no cause for complacency, considering the pervasive nature of acidic precipitation. Our study has provided a firm data base describing chemical and biological conditions in some Pennsylvania temporary ponds. We strongly suggest additional studies in other geographic regions, and a follow-up study in Pennsylvania to detect possible long term alterations.

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APPENDIX A

WATER CHEMISTRY OF AMPHIBIAN BREEDING PONDS IN CENTRAL PENNSYLVANIA

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Table A. Water chemistry characteristics of amphibian breeding ponds in Central Pennsylvania.

			(C)	(mg/L $CaCO_3$)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH_4	NO_3	SO_4	Cl	Tannin-lignin
Alan Seeger	3/28/84	4.29	8	-	0.64	0.26	1.83	0.30	-	-	-	-	1.29	0.43
	4/04/84	4.25	-	0	0.24	0.18	1.51	0.26	-	-	-	-	0.98	0.33
	4/11/84	4.60	11	-1.5	0.92	0.40	1.50	0.35	-	-	-	-	1.08	0.38
	4/25/84	4.45	9	0	0.46	0.37	1.91	0.51	-	-	-	-	1.40	0.27
	5/12/84	4.40	-	-	-	-	-	-	0.268	-	-	-	-	-
	6/22/84	4.49	-	-	-	-	-	-	0.175	-	-	-	-	-
Barrville 1	4/25/83	5.74	6	2.3	0.29	0.56	2.19	1.38	-	-	-	7.61	0.51	1.40
	5/02/83	5.75	13	-1.0	0.48	0.98	2.43	1.07	-	-	-	6.37	1.97	1.05
	5/09/83	6.15	9	12.0	0.43	0.67	4.05	2.23	-	-	-	4.31	0.93	1.72
	5/16/83	6.22	12	13.8	0.36	0.80	3.88	2.34	-	-	-	2.90	1.03	3.28
	5/24/83	6.05	14	9.3	0.39	0.54	2.94	1.77	-	-	-	4.14	0.44	1.33
	5/30/83	6.31	19	9.9	0.49	0.61	3.41	1.67	-	-	-	3.48	0.63	2.29
	6/07/83	6.37	11	16.6	0.65	0.82	5.19	2.44	-	-	-	2.36	0.81	3.66
	6/13/83	6.36	20	26.5	0.98	0.99	6.24	3.28	-	-	-	1.84	1.01	2.37
	6/20/83	6.24	18	34.0	1.54	1.74	10.75	5.29	-	-	-	5.35	2.21	2.56
	6/27/83	6.25	21	23.8	0.89	0.77	6.06	4.00	-	-	-	3.32	1.97	2.16
	3/28/84	5.68	11	-	0.45	0.37	3.58	1.94	-	-	-	-	1.05	1.45
	4/04/84	5.62	6	2.5	0.29	0.17	1.81	1.05	-	-	-	-	0.96	0.72
	4/11/84	5.74	17	5.0	0.58	0.57	2.40	1.31	-	-	-	-	1.17	1.39
	4/20/84	5.90	10	3.6	0.49	0.55	2.51	1.46	-	-	-	-	1.08	1.62
	5/12/84	5.30	-	-	-	-	-	-	0.074	-	-	-	-	-
	6/22/84	6.13	-	-	-	-	-	-	0.099	-	-	-	-	-
Barrville 2	3/20/83	4.96	7	-0.3	0.42	0.22	2.12	0.82	-	-	-	-	-	3.92
	3/21/83	4.85	5	-0.8	0.42	0.15	1.63	0.79	0.123	-	-	-	-	4.20
	3/28/83	5.01	2	-0.1	0.50	1.04	1.22	0.67	0.148	-	-	-	-	2.97
	4/04/83	4.94	5	-0.4	0.54	0.71	2.42	1.10	-	-	-	-	-	1.51
	4/11/83	4.72	6	-0.5	0.38	0.67	2.02	0.91	-	-	-	-	0.60	1.57
	4/18/83	4.91	6	0	0.42	0.79	2.07	0.88	-	-	-	-	1.03	2.19
	4/25/83	4.63	8	-0.6	0.25	0.75	2.03	0.86	-	-	-	10.69	0.72	1.67
	5/02/83	4.90	14	-0.9	0.35	1.09	2.70	0.83	-	-	-	8.84	1.32	1.93
	5/09/83	5.18	8	1.1	0.32	1.10	2.05	0.80	0.200	-	-	4.71	1.25	3.93
	5/16/83	5.12	12	0.5	0.29	1.28	2.32	0.90	-	-	-	5.52	1.49	5.08
	5/24/83	5.17	9	0	0.27	0.99	2.05	0.81	-	-	-	5.99	0.72	2.95

Table A (Continued)

			(C)	(mg/L CaCO$_3$)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH$_4$	NO$_3$	SO$_4$	Cl	Tannin-lignin
Barrville 2 (cont'd.)	5/30/83	5.43	19	1.3	0.36	1.41	2.52	0.70	–	–	–	3.38	1.14	4.39
	6/07/83	5.44	12	2.4	0.47	1.60	2.59	0.74	–	–	–	3.92	1.30	6.01
	6/13/83	5.77	20	3.3	0.58	1.92	2.96	0.68	–	–	–	2.02	1.90	6.15
	6/20/83	5.68	18	9.5	0.81	2.38	4.24	1.26	–	–	–	4.90	1.33	5.10
	6/27/83	5.22	21	1.3	1.16	2.14	2.76	1.10	–	–	–	11.08	0.74	3.67
	3/28/84	4.72	8	–	0.34	0.63	2.78	0.94	–	–	–	–	1.27	1.56
	4/04/84	4.60	3	–0.5	0.21	1.05	1.43	0.83	0.092	0.80	0.42	9.40	0.91	0.52
	4/05/84	4.50	4	–1.6	0.13	0.40	1.62	0.60	0.136	0.02	0.06	8.90	0.97	0.62
	4/11/84	5.27	17	1.1	0.60	1.02	2.41	0.84	0.111	0.14	0.11	2.70	1.55	2.60
	4/20/84	5.09	11	0.2	0.44	0.78	1.99	0.88	–	–	–	–	1.25	2.35
	6/22/84	5.02	–	–	–	–	–	–	0.202	–	–	–	–	–
Barrville 3	4/04/84	4.50	3	–1.5	0.27	0.45	1.58	0.74	0.115	0.10	0.50	8.00	1.07	0.66
	4/04/84	4.50	4	–1.6	0.23	0.46	1.30	0.65	0.144	0.02	0.13	8.80	1.05	0.66
	4/11/84	5.20	11	0.4	0.72	0.65	1.40	0.71	0.057	0.16	0.11	6.80	1.43	0.90
Bear Meadows	3/16/83	4.47	7	–0.8	0.61	0.69	2.05	0.27	–	–	–	14.00	–	3.14
	3/21/83	4.30	5	–0.3	0.50	0.61	2.07	0.46	0.184	–	–	17.81	–	2.83
	3/28/83	4.29	1	0	0.49	0.49	1.43	0.78	–	–	–	10.99	–	1.52
	4/04/83	4.16	4	0	0.67	0.80	2.51	1.32	–	–	–	18.59	–	2.12
	4/11/83	4.09	5	0	0.46	0.63	1.66	0.86	–	–	–	20.61	1.44	2.26
	4/18/83	4.12	4	0	0.42	0.44	2.55	0.95	–	–	–	14.91	0.90	1.73
	4/25/83	4.02	4	0	0.51	0.48	2.14	0.90	–	–	–	16.25	1.60	2.47
	5/02/83	4.09	4	0	0.28	0.74	2.26	0.74	–	–	–	13.30	1.26	2.37
	5/09/83	4.22	8	0	0.32	0.23	1.40	0.65	0.400	–	–	6.13	1.26	2.16
	5/16/83	4.26	11	0	0.33	0.21	1.36	0.64	–	–	–	5.72	1.02	3.18
	5/24/83	4.27	13	–0.3	0.28	0.17	1.77	0.81	–	–	–	7.11	0.59	2.87
	5/30/83	4.27	18	–0.8	0.34	0.19	1.55	0.54	–	–	–	7.09	0.78	2.70
	6/07/83	4.28	12	0	0.53	0.31	1.00	0.40	–	–	–	4.06	1.15	4.38
	6/13/83	4.47	17	–1.3	0.53	1.03	1.28	0.36	–	–	–	3.13	1.95	4.61
	6/20/83	4.27	18	0	0.54	0.85	0.73	0.62	–	–	–	1.84	2.53	5.10
	6/27/83	4.30	19	–0.3	0.55	0.46	0.63	0.52	–	–	–	6.42	2.08	4.71
	7/05/83	3.29	23	0	0.77	0.78	0.95	0.65	–	–	–	3.32	3.35	6.13
	7/11/83	4.73	23	–1.5	0.48	0.52	1.06	0.30	–	–	–	3.88	2.02	5.35
	7/18/83	4.64	23	3.5	0.46	0.98	1.05	0.36	–	–	–	3.06	3.15	6.11
	7/25/83	4.18	20	0	0.51	1.16	1.47	0.65	–	–	–	4.78	3.79	4.46
	8/02/83	4.35	22	–0.5	0.66	0.62	2.07	0.56	–	–	–	3.26	3.59	5.38

Table A (Continued)

Pond	Date	pH	(C) temp.	(mg/L CaCO₃) Alk.	Na	K	Ca	(mg/L) Mg	Al	NH_4	NO_3	SO_4	Cl	(mg/L tannic acid) Tannin-lignin
Bear Meadows (cont'd.)	3/28/84	4.24	4	-	0.44	0.19	1.49	0.55	-	-	-	-	1.19	1.27
	4/03/84	4.29	4	0	0.29	0.31	1.22	0.47	0.091	0.04	0.16	7.60	2.47	1.21
	4/04/84	4.10	2	0	0.29	0.35	0.97	0.63	0.142	0.07	0.51	8.60	1.33	1.39
	4/05/84	4.20	3	0	0.25	0.33	0.68	0.51	0.103	0.01	0.04	7.00	1.35	1.77
	4/11/84	4.20	9	0	0.44	0.65	1.50	0.71	0.124	0.02	0.10	11.90	1.21	1.55
	4/20/84	4.27	8	0	0.50	0.44	2.14	0.66	-	-	-	-	1.08	1.80
	4/25/84	4.39	-	0	0.43	0.33	1.04	0.68	-	-	-	-	1.09	1.71
	6/22/84	4.15	-	-	-	-	-	-	0.184	-	-	-	-	-
Centre Hall 5	9/24/82	6.81	15	655.5	10.14	164.14	34.13	13.43	-	-	3.10	14.32	47.94	10.58
Centre Hall 12	9/24/82	7.13	11	424.0	4.75	2.74	83.40	3.78	-	-	4.71	15.49	6.34	1.26
Centre Hall 13	9/24/82	7.61	18	4.5	2.29	0.97	46.91	4.46	-	-	0.41	8.06	17.06	0.87
Franklinville 2	9/24/82	5.44	16	1.8	0.13	1.08	1.14	0.14	-	-	0.07	3.76	0.73	0.67
Julian 1	9/18/82	6.84	11	154.2	0.39	1.58	13.69	7.48	-	-	0.87	1.81	1.89	0.90
	3/15/83	7.20	15	43.07	0.40	1.41	11.88	4.58	-	-	-	-	-	0
	3/21/83	7.19	6	33.0	0.07	0.91	7.58	3.87	0.028	-	-	-	-	1.08
	3/28/83	7.13	3	16.9	0.07	1.04	8.28	2.74	0.023	-	-	-	-	0.61
	4/04/83	7.01	9	34.6	0.13	1.26	11.06	3.51	-	-	-	-	-	0.83
	4/11/83	6.92	10	35.7	0.24	1.17	9.64	2.98	-	-	-	-	0.72	0.98
	4/18/83	6.90	9	37.2	0.23	1.10	12.11	3.23	-	-	-	-	1.14	1.02
	4/25/83	7.27	11	93.0	0.24	1.44	26.57	5.17	-	-	-	6.37	0.99	0.86
	5/02/83	7.08	16	41.8	0.20	1.30	14.60	3.17	-	-	-	3.91	1.14	0.74
	5/09/83	7.04	16	48.2	0.22	1.05	15.35	3.41	0.100	-	-	2.90	0.97	0.67
	5/16/83	6.90	14	48.7	0.14	1.00	14.90	3.80	-	-	-	2.50	0.72	0.75
	5/24/83	7.16	14	48.5	0.23	1.01	15.62	3.64	-	-	-	2.79	0.54	0.87
	5/30/83	7.21	17	50.1	0.10	0.69	16.07	3.94	-	-	-	2.70	0.41	0.90
	6/07/83	7.42	22	51.3	0.30	0.79	16.11	3.95	-	-	-	2.26	0.61	0.81
	6/13/83	7.56	28	57.0	0.23	0.82	17.82	5.02	-	-	-	6.46	0.73	0.94
	6/20/83	6.88	22	64.3	0.50	4.41	21.63	4.94	-	-	-	10.53	0.88	1.39
	6/27/83	6.78	21	61.2	0.30	1.00	16.38	6.88	-	-	-	1.53	0.90	1.32
	7/05/83	6.94	25	57.5	0.18	1.16	15.12	5.81	-	-	-	5.26	0.84	0.79
	7/11/83	7.04	20	67.0	0.39	1.92	18.03	6.63	-	-	-	3.43	1.57	1.14

Table A (Continued)

			(C)	(mg/L $CaCO_3$)	(mg/L)									(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH_4	NO_3	SO_4	Cl	Tannin-lignin
Julian 1 (cont'd.)	7/18/83	7.21	25	50.0	0.49	2.27	23.88	8.39	-	-	-	5.00	1.37	1.21
	7/25/83	7.22	22	64.5	0.27	2.24	18.62	7.00	-	-	-	7.80	1.11	5.40
	8/02/83	7.68	31	7.45	1.97	3.15	16.67	8.08	-	-	-	4.39	2.35	1.32
	3/28/84	6.87	[illegible]	-	0.19		19.19	2.73	-	-	-	-	-	0.63
	4/04/84	7.10	9	40.0	0.13	0.65	12.93	2.51	-	-	-	-	0.23	0.41
	4/11/84	7.00	7	44.7	0.52	0.63	14.71	2.98	-	-	-	-	1.29	0.79
	4/20/84	7.25	15	48.4	0.20	0.62	18.03	0.69	-	-	-	-	1.31	0.70
	5/12/84	7.50	-	-	-	-	-	-	0.019	-	-	-	-	-
	6/22/84	6.81	-	-	-	-	-	-	0.017	-	-	-	-	-
Julian 2	9/18/82	5.03	11	5.8	5.25	4.00	5.73	2.22	-	-	2.86	2.59	6.44	7.29
Julian 3	9/18/82	4.94	11	2.5	0.38	3.57	0.93	0.36	-	-	0.76	1.81	3.62	15.03
Julian 4	9/18/82	5.37	10	10.0	0.25	3.68	2.67	0.46	-	-	0.32	1.03	2.75	17.88
Julian 5	9/18/82	6.26	12	27.0	0.43	6.24	3.19	0.36	-	-	1.44	2.59	3.46	70.12
	3/28/84	5.30	10	-	0.42	1.55	2.19	0.45	-	-	-	-	1.29	1.47
	4/03/84	5.20	7	1.5	0.13	1.53	1.89	0.34	0.072	0.04	0.22	6.40	1.35	1.30
	4/04/84	5.30	8	1.0	0.17	1.54	2.45	0.31	0.080	0.06	1.00	5.40	1.29	1.31
	4/05/84	5.20	9	1.3	0.17	1.27	1.22	0.29	0.066	0.02	0.47	5.90	1.06	1.41
	4/11/84	5.40	8	2.9	0.32	1.60	1.81	0.38	0.024	0.07	0.51	6.60	1.17	1.55
	4/20/84	5.60	12	0.8	0.22	1.83	1.99	0.36	-	-	-	-	1.26	1.43
	4/25/84	5.45	10	0.2	0.40	1.80	1.59	0.43	0.076	-	-	-	1.15	1.25
	6/22/84	5.68	-	-	-	-	-	-	-	-	-	-	-	-
Julian 6	3/14/83	4.72	9	−0.8	0.56	1.07	2.45	0.83	-	-	-	-	-	6.16
	3/21/83	4.86	5	−1.3	0.48	0.36	1.58	0.64	0.196	-	-	-	-	5.15
	3/28/83	4.61	3	−0.5	0.44	0.73	0.90	0.42	0.199	-	-	-	-	1.23
	4/04/83	4.75	7	−0.9	0.55	0.81	1.27	0.82	-	-	-	-	-	1.71
•	4/11/83	4.40	7	−0.7	0.38	0.75	1.73	0.47	-	-	-	-	2.19	1.28
	4/18/83	4.71	8	−0.7	0.36	0.63	1.22	0.54	-	-	-	-	0.80	1.56
	4/24/83	-	8	-	0.37	0.58	1.64	0.53	-	-	-	5.14	1.38	1.14
•	5/02/83	4.36	14	−1.0	0.37	0.67	1.17	0.39	-	-	-	5.26	1.21	2.22
	5/09/83	4.75	14	−1.4	0.33	0.71	1.11	0.43	0.300	-	-	3.30	1.00	1.59
	5/16/83	4.62	12	−1.1	0.34	0.82	1.10	0.44	-	-	-	2.90	1.35	2.45

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Table A (Continued)

Pond	Date	pH	(C) temp.	(mg/L $CaCO_3$) Alk.	Na	K	Ca	(mg/L) Mg	Al	NH_4	NO_3	SO_4	Cl	(mg/L tannic acid) Tannin-lignin
Julian 6 (cont'd.)	5/24/83	4.84	13	−0.9	0.54	0.74	1.18	0.42	-	-	-	3.96	0.55	2.29
	5/30/83	4.94	15	−0.5	0.51	0.88	1.20	0.36	-	-	-	2.18	0.89	1.75
	6/07/83	4.94	18	−1.6	0.36	0.61	1.24	0.40	-	-	-	2.60	0.74	1.81
	6/13/83	5.08	20	−0.3	0.48	0.92	1.50	0.38	-	-	-	3.13	2.12	2.14
	6/20/83	4.91	19	1.0	0.42	1.32	1.45	0.30	-	-	-	3.50	1.29	3.65
	6/27/83	4.89	18	−0.2	0.72	1.91	1.27	0.45	-	-	-	4.48	2.20	3.49
	7/05/83	5.21	21	-	0.72	1.36	1.16	0.58	-	-	-	1.53	1.53	4.18
	7/11/83	5.27	15	0.3	1.26	1.50	1.45	0.64	-	-	-	2.07	-	5.56
	7/18/83	5.14	22	0.8	1.39	1.49	1.11	0.37	-	-	-	2.19	2.10	5.66
	7/25/83	5.02	19	0.3	0.73	0.68	1.92	1.26	-	-	-	2.62	1.69	6.14
	8/02/83	5.00	22	−0.3	0.90	1.56	1.98	0.34	-	-	-	3.04	2.73	8.40
	3/28/84	4.62	8	-	0.42	0.62	1.16	0.46	-	-	-	-	1.44	1.80
	4/03/84	4.59	5	−0.5	0.25	0.63	1.19	0.38	0.167	0.05	0.13	5.80	1.08	0.98
	4/04/84	4.70	6	−2.5	0.28	0.72	1.04	0.38	0.157	0.01	0.09	5.10	1.21	1.15
	4/05/84	4.50	8	−1.5	0.27	0.52	1.30	0.35	0.146	0.02	0.10	5.40	1.15	1.21
	4/11/84	4.79	7	−0.9	0.47	0.80	1.23	0.43	0.146	0.05	0.31	5.90	1.15	1.25
	4/20/84	4.80	11	−1.3	0.35	0.83	1.09	0.41	-	-	-	-	1.08	0.94
	4/25/84	4.85	11	−0.8	0.56	0.93	1.02	0.51	-	-	-	-	1.31	1.00
	6/22/84	4.81	-	-	-	-	-	-	0.102	-	-	-	-	-
Julian 8	3/15/83	5.42	9	1.2	0.28	2.36	2.87	0.36	-	-	-	-	-	5.77
	3/21/83	5.44	5	1.0	0.17	0.80	1.58	0.61	-	-	-	-	-	5.62
	3/28/83	5.27	5	1.4	0.29	2.17	2.07	0.44	-	-	-	-	-	2.96
	4/04/83	5.20	7	−0.2	0.18	1.67	2.31	0.63	-	-	-	-	-	3.10
	4/11/83	5.13	7	0.1	0.19	1.53	1.87	0.28	-	-	-	-	1.22	3.67
	4/18/83	4.70	8	−0.7	0.19	1.51	1.99	0.54	-	-	-	-	1.07	3.57
	4/24/83	4.80	9	−1.0	0.10	1.51	2.50	0.50	-	-	-	8.84	1.12	3.08
	5/02/83	5.11	14	−0.5	0.18	1.33	2.07	0.46	-	-	-	5.76	1.15	3.69
	5/09/83	5.28	15	1.4	0.18	1.52	2.03	0.51	0.300	-	-	3.51	1.43	4.06
	5/16/83	5.00	13	0.6	0.12	1.54	1.91	0.51	-	-	-	3.51	1.37	4.77
	5/24/83	5.35	15	1.1	0.17	1.56	1.86	0.48	-	-	-	3.33	0.85	4.19
	5/30/83	5.50	12	2.0	0.21	1.87	2.44	0.51	-	-	-	2.54	1.19	5.63
	6/07/83	5.56	20	1.7	0.36	1.72	2.73	0.58	-	-	-	2.55	1.43	4.35
	6/13/83	5.59	24	3.0	0.99	2.07	2.33	0.39	-	-	-	3.50	1.97	4.00
	6/20/83	5.40	20	1.5	0.20	1.67	2.29	0.46	-	-	-	4.61	1.06	4.43
	6/27/83	5.28	20	3.1	0.44	1.52	2.53	0.55	-	-	-	5.26	1.09	3.55

Table A (Continued)

			(C)	(mg/L CaCO$_3$)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH$_4$	NO$_3$	SO$_4$	Cl	Tannin-lignin
Julian 8 (cont'd.)	7/05/83	5.27	22	2.3	0.40	1.61	2.27	0.71	-	-	-	5.65	1.10	3.58
	7/11/83	5.58	19	3.8	0.47	1.73	2.80	0.81	-	-	-	2.75	1.50	4.48
	7/18/83	5.57	22	4.0	0.47	1.78	3.12	0.50	-	-	-	9.53	1.32	4.56
	7/25/83	5.39	20	2.5	0.52	1.80	3.77	0.84	-	-	-	10.39	1.20	3.32
	8/02/83	5.43	33	0.4	0.70	1.53	3.10	0.37	-	-	-	3.94	1.85	4.28
	3/28/84	4.78	1	-	0.11	1.53	1.46	0.30	-	-	-	-	0.95	2.01
	4/04/84	5.10	6	0.5	0.29	1.80	1.61	0.32	-	-	-	-	1.07	2.48
	4/11/84	5.33	12	2.0	0.39	2.20	1.71	0.38	-	-	-	-	1.65	3.11
	4/20/84	5.34	11	0.2	0.11	2.29	1.96	0.36	-	-	-	-	1.38	2.69
	4/25/84	5.40	9	1.7	0.12	2.22	1.47	0.44	-	-	-	-	1.42	2.85
	5/12/84	5.80	-	-	-	-	-	-	0.112	-	-	-	-	-
	6/22/84	5.63	-	-	-	-	-	-	0.085	-	-	-	-	-
Julian 10	9/21/82	8.55	18	67.2	4.14	3.32	13.24	3.32	-	-	1.02	6.89	1.62	0.46
Julian 11	9/21/82	7.42	16	151.0	3.27	2.24	33.83	3.19	-	-	0	6.89	3.31	1.59
Julian 12	9/21/82	6.56	16	60.5	3.39	3.80	19.03	4.32	-	-	0.24	45.99	4.53	2.30
Julian 13	9/24/82	6.95	12	183.3	8.12	11.99	29.34	2.74	-	-	0.46	9.63	7.89	0.37
	3/14/83	7.63	9	81.8	2.46	4.43	24.16	2.47	-	-	-	9.56	-	0.94
	3/21/83	7.81	6	50.9	3.35	3.06	25.87	3.23	0.530	-	-	-	-	0.60
	3/28/83	7.41	2	60.7	3.68	4.50	21.46	3.13	0.311	-	-	10.99	-	0.91
	4/04/83	7.54	9	52.6	4.00	4.21	19.18	3.17	-	-	-	6.51	-	0.86
	4/11/83	7.28	7	59.2	3.14	3.36	17.75	2.68	-	-	-	4.53	3.68	1.02
	4/18/83	7.41	7	63.5	4.19	3.81	19.88	3.19	-	-	-	2.90	3.80	1.14
	4/25/83	7.07	8	46.8	4.28	3.74	15.18	3.10	-	-	-	8.23	5.56	1.35
	5/02/83	7.09	16	45.4	4.25	3.80	20.06	2.83	-	-	-	6.37	6.43	1.61
	5/09/83	6.99	14	51.5	4.40	3.77	15.91	3.03	0.600	-	-	4.92	6.47	1.63
	5/16/83	6.96	12	63.0	5.78	4.04	19.98	3.37	-	-	-	3.71	6.87	1.67
	5/24/83	7.40	13	84.2	3.03	4.40	22.87	2.81	-	-	-	6.66	3.62	2.02
	5/30/83	7.35	15	77.4	3.07	4.42	30.27	2.86	-	-	-	2.91	2.49	1.92
	6/07/83	7.19	16	96.8	4.20	5.26	26.40	3.81	-	-	-	2.26	7.15	2.12
	6/13/83	7.25	22	111.3	5.22	0.65	28.66	4.05	-	-	-	2.39	7.19	1.85
	6/20/83	7.17	19	68.5	0.23	2.39	19.85	6.18	-	-	-	2.02	19.39	0.97
	6/27/83	6.93	21	112.0	24.36	5.64	28.22	8.40	-	-	-	13.41	37.97	1.12
	7/05/83	7.36	22	83.0	7.03	5.16	22.49	4.54	-	-	-	9.14	9.10	1.37
	7/11/83	7.12	-	111.1	1.38	4.27	27.09	5.22	-	-	-	7.06	11.07	1.81
	7/18/83	7.17	23	70.3	7.26	5.90	30.45	5.06	-	-	-	4.78	13.82	1.82

Table A (Continued)

			(C)	(mg/L CaCO3)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH_4	NO_3	SO_4	Cl	Tannin-lignin
Julian 14	3/16/83	4.95	9	-0.4	0.66	0.84	2.10	0.98	-	-	-	-	-	7.31
	3/21/83	4.67	6	-1.8	0.53	0.25	1.25	0.68	0.149	-	-	-	-	3.79
	3/28/83	4.67	1	-1.0	0.50	0.99	1.12	0.64	0.113	-	-	-	-	1.70
	4/04/84	4.94	5	-0.4	0.60	0.87	1.84	0.98	-	-	-	-	-	1.51
*	4/11/83	4.53	7	-1.5	0.51	0.85	1.32	0.78	-	-	-	-	0.11	1.43
	4/18/83	4.78	7	-1.1	0.65	0.76	1.30	0.75	-	-	-	-	1.42	1.76
	4/25/83	4.60	7	-1.1	1.04	0.78	1.91	0.76	-	-	-	10.08	2.05	2.10
	5/02/83	4.78	14	-1.6	0.56	0.80	1.68	0.68	-	-	-	7.61	1.43	1.96
	5/09/83	4.81	11	-0.2	0.59	0.85	1.06	0.64	0.300	-	-	3.91	1.41	1.92
	5/16/83	5.65	12	-1.6	0.59	1.11	1.41	0.59	-	-	-	4.11	1.67	4.20
	5/24/83	5.05	13	-0.2	0.60	1.12	1.36	0.63	-	-	-	2.97	1.01	4.16
	5/30/83	5.08	15	-0.2	0.64	1.31	1.46	0.53	-	-	-	2.91	1.30	4.53
	6/07/83	5.19	18	3.3	0.61	1.81	1.33	0.56	-	-	-	2.89	2.52	6.01
	6/13/83	5.35	18	0.8	0.64	2.02	1.48	0.52	-	-	-	3.69	0.83	6.10
	6/20/83	5.11	18	0	0.91	1.82	0.93	0.22	-	-	-	1.47	2.34	6.65
	6/27/83	5.06	23	0.5	1.69	1.91	1.59	0.45	-	-	-	4.48	5.13	3.41
Julian 20	3/28/84	4.62	3	-	0.31	1.81	1.94	0.49	-	-	-	-	2.16	2.77
	4/04/84	4.65	8	-0.5	0.29	1.67	1.49	0.43	-	-	-	-	1.86	1.78
	4/11/84	4.60	7	-0.3	0.37	2.52	1.90	0.49	-	-	-	-	2.22	2.93
	4/20/84	4.62	12	0	0.30	2.56	1.91	0.48	-	-	-	-	2.13	2.58
	5/12/84	4.70	-	-	-	-	-	-	0.249	-	-	-	-	-
	6/22/84	4.95	-	-	-	-	-	-	0.205	-	-	-	-	-
Madisonburg 1	10/24/82	6.40	9	30.0	1.39	1.67	0.02	0.90	-	-	-	-	2.02	0.28
Madisonburg 2	10/24/82	5.70	7	4.5	0.02	0.59	0.01	0.60	-	-	-	-	0.65	0
Madisonburg 3	10/24/82	6.55	9	29.0	0.10	1.21	0.01	1.06	-	-	-	-	1.43	0
McAlevy 1	3/14/83	4.32	11	0	0.48	0.27	1.84	0.74	-	-	-	-	-	0.70
	3/21/83	4.39	5	-0.7	0.48	0.05	1.14	0.65	0.530	-	-	-	-	0.40
	3/28/83	4.32	3	-0.9	0.56	0.46	0.96	0.43	0.510	-	-	-	-	0.48
	4/04/83	4.30	6	-0.7	0.42	0.42	1.41	0.64	-	-	-	-	-	0.51
	4/11/83	4.24	6	0	0.44	0.59	1.21	0.46	-	-	-	-	0.79	0.72
	4/18/83	4.30	6	-0.5	0.58	0.62	1.31	0.59	-	-	-	-	0.75	0.64
	4/25/83	4.17	7	0	0.35	0.44	1.24	0.48	-	-	-	11.31	0.77	0.60

Table A (Continued)

			(C)	(mg/L CaCO$_3$)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH$_4$	NO$_3$	SO$_4$	Cl	Tannin-lignin
McAlevy 1 (cont'd.)	5/02/83	4.37	15	-0.8	0.90	0.60	1.42	0.48	-	-	-	12.42	3.10	0.81
	5/09/83	4.33	15	-0.5	0.53	0.68	0.87	0.54	0.800	-	-	7.53	1.19	0.51
	5/16/83	4.32	12	-1.1	0.53	0.47	1.34	0.49	-	-	-	5.52	0.97	0.41
	5/24/83	4.33	18	-0.7	0.38	0.69	1.00	0.41	-	-	-	8.06	0.45	0.82
	5/30/83	4.44	20	-1.4	0.71	0.76	1.07	0.37	-	-	-	6.82	0.70	0.64
	6/07/83	4.39	17	-1.1	0.74	0.81	1.31	0.48	-	-	-	6.89	1.03	0.65
	6/13/83	4.36	20	-0.7	0.50	0.86	1.45	0.49	-	-	-	6.09	0.90	0.91
	6/20/83	4.52	19	-0.6	0.35	2.11	0.88	0.26	-	-	-	4.61	1.18	1.24
	3/28/84	4.32	2	-	0.40	0.24	1.25	0.48	-	-	-	-	1.06	0.34
	4/04/84	4.20	6	0	0.29	0.60	1.01	0.35	0.600	0.03	0.25	10.00	1.12	0.52
	4/04/84	4.20	8	0	0.27	0.56	0.78	0.37	0.600	0.01	0.09	10.70	0.98	0.56
	4/11/84	4.40	16	-2.1	0.95	1.06	1.26	0.53	0.600	0.03	0.08	11.40	1.43	0.71
	4/20/84	4.44	12	0	0.51	0.64	1.07	0.51	-	-	-	-	1.09	0.33
	4/25/84	4.40	12	0	0.47	0.53	1.07	0.64	-	-	-	-	1.18	0.29
	6/22/84	4.20	-	-	-	-	-	-	0.482	-	-	-	-	-
McAlevy 2	3/15/83	4.43	13	-0.5	0.65	1.09	2.35	0.81	-	-	-	-	-	1.08
	3/21/83	4.38	5	-0.1	0.49	0.19	1.47	0.52	-	-	-	-	-	0.77
	3/28/83	4.44	3	-1.5	0.57	0.89	1.57	0.69	0.300	-	-	-	-	0.63
	4/04/83	4.35	5	-1.4	0.38	0.93	1.74	0.60	0.490	-	-	-	-	0.73
	4/11/83	4.37	6	-0.9	0.43	1.01	2.02	0.75	-	-	-	-	0.94	0.74
	4/18/83	4.41	5	0	0.89	1.30	1.64	0.71	-	-	-	-	0.85	0.67
	4/25/83	4.11	7	0	0.35	1.02	1.97	0.67	-	-	-	7.61	0.83	0.62
	5/02/83	4.45	15	-1.7	0.31	1.23	1.37	0.74	-	-	-	13.16	1.06	0.50
	5/09/83	4.44	10	-0.8	0.44	1.16	1.90	0.66	-	-	-	7.53	1.31	0.69
	5/16/83	4.51	11	-1.8	0.41	1.30	1.44	0.60	-	-	-	5.52	1.23	1.27
	5/24/83	4.54	15	-1.9	1.14	1.20	1.65	0.68	-	-	-	12.88	0.62	0.76
	5/30/83	4.52	20	-2.2	0.64	0.64	1.49	0.46	-	-	-	7.56	0.77	0.90
	6/07/83	4.46	16	-1.4	0.64	1.43	1.00	0.44	-	-	-	5.82	0.92	0.93
	6/13/83	4.67	20	-1.4	0.47	1.94	1.36	0.33	-	-	-	5.91	1.14	1.64
	6/20/83	4.55	18	-2.25	0.52	1.87	0.90	0.25	-	-	-	4.24	1.24	1.97
	3/28/84	4.42	2	-	0.44	0.79	1.85	0.62	-	-	-	-	1.27	0.33
	4/04/84	4.40	4	-0.5	0.29	1.05	1.46	0.63	-	-	-	-	1.04	0.35
	4/11/84	4.50	16	-2.3	0.97	1.57	1.73	0.74	-	-	-	-	1.56	0.77
	4/20/84	4.44	12	0	0.46	1.19	1.56	0.66	-	-	-	-	1.18	0.43
	4/25/84	4.47	12	0	0.47	1.13	1.56	0.75	-	-	-	-	1.31	0.35
	5/12/84	4.60	-	-	-	-	-	-	0.350	-	-	-	-	-
	6/22/84	4.39	-	-	-	-	-	-	0.311	-	-	-	-	-

Table A (Continued)

			(C)	(mg/L CaCO$_3$)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH$_4$	NO$_3$	SO$_4$	Cl	Tannin-lignin
McAlevy 3	4/11/84	4.50	16	-2.4	0.92	1.33	1.78	0.55	-	-	-	-	1.55	1.50
McAlevy 6	3/20/83	4.41	5	-0.5	0.42	0.16	1.60	0.68	-	-	-	-	-	1.12
	3/21/83	4.19	5	0	0.41	0.12	1.49	0.64	0.320	-	-	-	-	2.04
	3/28/83	4.54	2	-2.0	0.46	0.55	1.22	0.64	0.248	-	-	-	-	0.32
	4/04/83	4.41	4	-1.1	0.35	0.56	1.66	0.80	-	-	-	-	-	0.65
	4/11/83	4.23	7	0	0.41	0.63	1.18	0.61	-	-	-	-	0.98	1.38
	4/18/83	4.30	6	-0.4	0.36	0.56	1.52	0.69	-	-	-	-	0.77	0.64
	4/25/83	4.13	6	0	0.25	0.58	1.22	0.52	-	-	-	8.23	0.70	1.14
	5/02/83	4.34	14	-1.0	0.36	0.68	1.80	0.71	-	-	-	8.84	0.74	1.04
	5/09/83	4.52	6	-1.5	0.33	0.65	1.45	0.64	0.500	-	-	5.52	0.82	0.64
	5/16/83	4.15	9	0	0.38	0.49	1.07	0.63	-	-	-	3.91	0.70	1.30
	5/24/83	4.41	13	-1.8	0.29	0.73	1.33	0.55	-	-	-	4.41	0.53	1.39
	5/30/83	4.44	18	-1.5	0.56	0.56	1.15	0.45	-	-	-	5.41	0.61	1.27
	6/07/83	4.60	11	-0.7	0.51	0.61	0.98	0.47	-	-	-	3.67	0.73	1.31
	6/13/83	5.25	19	3.4	0.67	0.90	1.70	0.44	-	-	-	4.24	2.27	9.18
McAlevy 7	4/11/83	4.20	5	0	0.29	0.55	1.19	0.49	-	-	-	12.89	0.68	1.58
	4/18/83	4.24	6	0	0.41	0.48	1.23	0.61	-	-	-	10.11	0.79	1.21
	4/25/83	4.05	6	0	0.23	0.49	1.06	0.42	-	-	-	11.31	0.74	1.13
	5/02/83	4.37	14	-1.0	0.51	1.84	1.38	0.46	-	-	-	11.93	2.63	1.59
	5/09/83	4.54	7	-1.6	0.24	0.56	1.14	0.56	0.500	-	-	5.32	0.86	1.06
	5/16/83	4.40	11	-1.1	0.30	0.69	1.14	0.59	-	-	-	3.30	0.87	1.48
	5/24/83	4.46	13	-1.5	0.27	0.68	1.05	0.45	-	-	-	4.14	0.59	1.61
	5/30/83	4.58	19	-1.9	0.54	0.54	1.22	0.45	-	-	-	3.54	0.63	1.15
	6/07/83	4.61	11	-7.8	0.59	0.81	1.03	0.43	-	-	-	3.43	0.83	1.65
	6/13/83	4.73	20	-0.2	0.44	0.60	1.03	0.22	-	-	-	3.87	1.46	3.58
	6/20/83	4.90	17	-0.9	0.96	1.49	0.71	0.20	-	-	-	3.50	2.29	6.75
	6/27/83	4.72	20	-0.5	1.29	1.03	0.96	0.42	-	-	-	4.48	1.92	4.07
	7/05/83	4.54	23	-1.5	1.61	1.52	1.30	0.60	-	-	-	4.48	2.94	3.28
	3/28/84	4.29	5	-	0.40	0.32	1.65	0.65	-	-	-	-	0.99	0.49
	4/03/84	4.50	2	-0.5	0.27	0.49	1.24	0.51	0.300	0.07	0.13	9.2	0.82	0.34
	4/04/84	4.30	2	-0.5	0.16	0.27	0.63	0.38	0.400	0.07	0.44	7.6	0.96	0.41
	4/05/84	4.30	3	-0.6	0.19	0.27	0.68	0.36	0.400	0.03	0.17	7.6	0.99	0.67
	4/11/84	4.69	17	-1.5	0.46	0.84	1.12	0.39	0.400	0.04	0.08	8.8	1.19	1.63
	4/20/84	4.19	9	0	0.48	0.75	1.61	0.66	-	-	-	-	1.29	0.70
	4/25/84	4.50	11	0	0.56	0.82	1.09	-	-	-	-	-		0.55
	6/22/84	4.35	-	-	-	-	-	-	0.279	-	-	-	-	-

Table A (Concluded)

			(C)	(mg/L CaCO$_3$)				(mg/L)						(mg/L tannic acid)
Pond	Date	pH	temp.	Alk.	Na	K	Ca	Mg	Al	NH$_4$	NO$_3$	SO$_4$	Cl	Tannin-lignin
Mingoville 1	10/22/82	6.33	7	29.0	1.73	3.09	0.03	1.43	-	-	-	-	2.72	0.05
Mingoville 2	10/22/82	7.07	9	82.5	2.52	2.27	0.06	1.63	-	-	-	-	2.46	0.11
Mingoville 3	10/22/82	6.16	8	29.8	1.86	2.63	0.02	1.39	-	-	-	-	2.18	0
Mingoville 4	9/22/82	7.02	11	22.2	0.01	2.31	0.03	0.97	-	-	-	-	2.94	0
Mingoville 5	9/22/82	5.52	12	42.8	2.05	6.98	0.02	0.74	-	-	-	-	4.67	7.50
Port Matilda 1	9/21/82	5.88	9	10.0	1.42	2.24	2.74	1.00	-	-	0.33	1.03	2.27	3.81
Port Matilda 2	9/21/82	5.52	18	5.2	0.93	1.87	0.24	0.26	-	-	-	3.76	1.60	1.32
State College 1	9/24/82	7.40	17	464.5	4.51	4.23	65.50	17.42	-	-	4.64	22.53	6.30	1.42
Woodward 1	10/24/82	6.68	9	35.5	2.57	0.95	0.02	0.75	-	-	-	-	2.54	0

APPENDIX B

pH'S OF TEMPORARY PONDS IN THE DELAWARE WATER GAP NATIONAL RECREATION AREA

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Table B. pH's of 36 temporary ponds in the Delaware Water Gap National Recreation Area (measured between March 20 and April 1, 1985.

Pond Name		pH	Pond Name		pH
Buskill	1	5.97	Milford	1	5.05
	2	6.54		2	5.95
	3	5.65		3	5.37
				4	6.46
Culver's Gap	1	5.00		5	5.01
				6	4.44
Lake Maskenozha	1	6.35		7	5.91
	2	3.80		8	6.43
	3	5.20		9	4.90
	4	4.30		10	5.68
	5	5.92		11	6.43
	6	5.91		12	6.43
	7	4.16		13	6.43
	8	6.37		14	6.43
	9	5.67		15	6.43
	10	5.50		16	6.43
	11	6.07		17	6.43
	12	6.13		18	5.85
	13	4.90			
			Stroudsburg	1	5.97

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APPENDIX C

GEOGRAPHIC COORDINATES OF AMPHIBIAN BREEDING PONDS IN PENNSYLVANIA

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Table C: Geographic Coordinates of Amphibian Breeding Ponds in Pennsylvania Which Were Chemically Analyzed.

Pond		Latitude	Longitude
Alan Seeger		40° 43' 00" N	77° 41' 29" W
Barrville	1	40° 44' 36" N	77° 44' 08" W
	2	40° 44' 36" N	77° 44' 02" W
	3	40° 44' 36" N	77° 43' 53" W
Bear Meadows		40° 43' 54" N	77° 45' 13" W
Buskill	1	41° 03' 49" N	75° 08' 54" W
	2	41° 04' 11" N	75° 08' 34" W
	3	41° 04' 14" N	75° 08' 34" W
Centre Hall	5	40° 49' 45" N	77° 43' 41" W
	12	40° 46' 47" N	77° 40' 27" W
	13	40° 47' 53" N	77° 39' 21" W
Culver's Gap	1	41° 14' 00" N	74° 52' 04" W
Franklinville	2	40° 44' 09" N	78° 04' 00" W
Julian	1	40° 47' 46" N	77° 57' 10" W
	2	40° 47' 35" N	77° 58' 11" W
	3	40° 47' 48" N	77° 57' 59" W
	4	40° 47' 52" N	77° 57' 45" W
	5	40° 46' 27" N	77° 56' 59" W
	6	40° 46' 27" N	77° 57' 02" W
	8	40° 47' 27" N	77° 55' 40" W
	10	40° 49' 00" N	77° 59' 12" W
	11	40° 49' 10" N	77° 58' 49" W
	12	40° 47' 56" N	77° 59' 20" W
	13	40° 49' 28" N	77° 54' 12" W
	14	40° 47' 38" N	77° 58' 02" W
	20	40° 48' 29" N	77° 56' 30" W
Lake Maskenozha	1	41° 12' 58" N	74° 52' 32" W
	2	41° 13' 05" N	74° 53' 37" W
	3	41° 14' 35" N	74° 52' 57" W
	4	41° 14' 30" N	74° 53' 08" W
	5	41° 14' 40" N	74° 52' 50" W
	6	41° 14' 42" N	74° 52' 53" W
	7	41° 08' 13" N	74° 57' 05" W
	8	41° 08' 09" N	74° 56' 32" W
	9	41° 08' 11" N	74° 56' 26" W
	10	41° 08' 17" N	74° 56' 17" W
	11	41° 08' 36" N	74° 55' 55" W
	12	41° 08' 38" N	74° 55' 45" W
	13	41° 08' 48" N	74° 55' 55" W

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Table C (Concluded)

Pond		Latitude	Longitude
Madisonburg	1	40° 53' 30" N	77° 36' 35" W
	2	40° 53' 26" N	77° 34' 35" W
	3	40° 54' 32" N	77° 34' 27" W
McAlevy	1	40° 43' 38" N	77° 51' 02" W
	2	40° 43' 41" N	77° 50' 49" W
	3	40° 43' 40" N	77° 50' 55" W
	6	40° 43' 45" N	77° 45' 22" W
	7	40° 43' 39" N	77° 45' 33" W
Milford	1	41° 16' 55" N	74° 50' 11" W
	2	41° 17' 25" N	74° 50' 21" W
	4	41° 17' 22" N	74° 51' 45" W
	5	41° 17' 10" N	74° 51' 52" W
	6	41° 17' 14" N	74° 51' 53" W
	7	41° 15' 27" N	74° 52' 00" W
	8	41° 16' 22" N	74° 51' 58" W
	9	41° 16' 05" N	74° 52' 00" W
	10	41° 16' 12" N	74° 52' 14" W
	11	41° 17' 47" N	74° 51' 00" W
	12	41° 18' 38" N	74° 48' 33" W
	13	41° 18' 38" N	74° 48' 33" W
	14	41° 18' 38" N	74° 48' 33" W
	15	41° 18' 38" N	74° 48' 33" W
	16	41° 18' 38" N	74° 48' 33" W
	17	41° 18' 38" N	74° 48' 33" W
	18	41° 18' 25" N	74° 48' 27" W
Mingoville	1	40° 54' 08" N	77° 40' 02" W
	2	40° 54' 45" N	77° 39' 30" W
	3	40° 54' 40" N	77° 39' 32" W
	4	40° 55' 33" N	77° 42' 54" W
	5	40° 56' 22" N	77° 39' 45" W
Port Matilda	1	40° 45' 53" N	78° 01' 00" W
	2	40° 45' 52" N	78° 00' 58" W
State College	1	40° 48' 44" N	77° 00' 58" W
Stroudsburg	1	40° 58' 28" N	75° 08' 27" W
Woodward	1	40° 58' 47" N	77° 18' 26" W

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16. Abstract (Limit: 200 words)

This study assessed the impacts of acid deposition on amphibians breeding in temporary ponds in Pennsylvania by investigating the lowest pH's at which embryos could hatch, the physiological effects of low pH on amphibian larvae, pond chemistry and the influence of rainfall on pond pH, and the effect of pond pH on embryonic survival and local distribution of *Ambystoma jeffersonianum* and *Rana sylvatica*. At very low pH's, embryos stopped development soon after exposure. At higher but still lethal pH's, embryos became curled and failed to hatch. Embryos of *Ambystoma* were able to hatch even though they were curled, but *R. sylvatica* became trapped and died. Acute exposure to low pH's depressed sodium influx and accelerated sodium efflux, with a net loss of 50% of body sodium resulting in death. Increasing the external calcium concentration extended survival time by slowing the loss of sodium. Chronic exposure to low pH's resulted in reduction in body sodium but to a lesser degree. *R. sylvatica* tadpoles from a low pH pond had lower body sodium than tadpoles from a nearby high pH pond. Tadpoles from both ponds placed in a low pH pond underwent higher sodium efflux than when placed in the high pH pond. In studying the effect of low environmental pH, *A. jeffersonianum* was intolerant of low pH and was absent from most acidic ponds. *R. sylvatica* was tolerant and was found in ponds with the lowest pH.

17. Document Analysis a. Descriptors

Acidification, pH, *Rana sylvatica*, *Ambystoma jeffersonianum*, amphibians, sodium efflux, sodium influx, Pennsylvania

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Acid rain, Jefferson salamanders, wood frogs, temporary ponds, distribution, reproductive success, embryo survival

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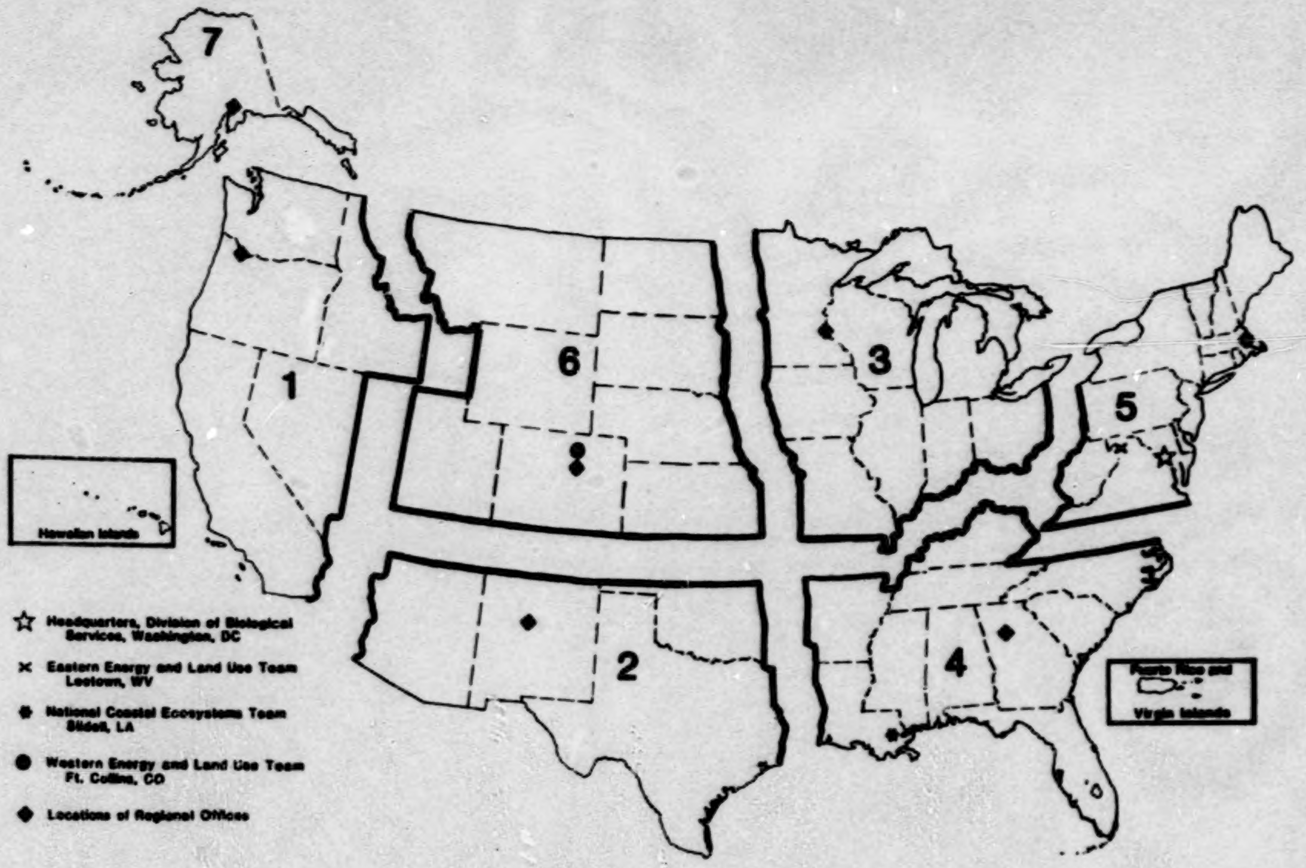
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